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(56) Related Art  
**Johnson et al, J. Infect. Dis. 1997, Vol. 176, No. 5, pages 1215-1224**  
**Johnson et al, J. Infect. Dis. 1997, Vol. 176, 1215-1224.**  
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**US 5824307**  
**Subramanian et al, Pediatr. Infect. Dis. J. 1998, Vol. 17, No. 2**  
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(54) Title: METHODS OF ADMINISTERING/DOSING ANTI-RSV ANTIBODIES FOR PROPHYLAXIS AND TREATMENT

A

DIQMTQSPST LSASVGDRVT ITCKCOLSVGYMH WYQQKPG 40  
CDR L1

KAPKLLIY DTSKLAS GVPSR FSGSGSGTEF TLTSSLOPD 80  
CDR L2

DFATYYC FQSGGYPT FGGGTKLEIK 106  
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLT TSGMSVG WIR 40  
CDR H1

QPPGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80  
CDR H2

VLKVTNMDPA DTATYYCAR SMITNWFYDV W GAGTTVTVSS 120  
CDR H3

(57) Abstract: The present invention encompasses novel antibodies and fragments thereof which immunospecifically bind to one or more RSV antigens and compositions comprising said antibody fragments. The present invention encompasses methods preventing respiratory syncytial virus (RSV) infection in a human, comprising administering to said human a prophylactically effective amount of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, wherein a certain serum titer of said antibodies or antibody fragments is achieved in said human subject. The present invention also encompasses methods for treating or ameliorating symptoms associated with a RSV infection in a human, comprising administering to said human a therapeutically effective amount of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, wherein a certain serum titer of said antibodies or antibody fragments is achieved in said human subject. The present invention further encompasses compositions comprising

antibodies or fragments thereof that immunospecifically bind to a SRV antigen, and methods using said compositions for detection or diagnosis a RSV infection.



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**METHODS OF ADMINISTERING/DOSING  
ANTI-RSV ANTIBODIES FOR  
PROPHYLAXIS AND TREATMENT**

5           **1.     INTRODUCTION**

The present invention relates to compositions comprising antibodies or fragments thereof that immunospecifically bind to a RSV antigen and methods for preventing, treating or ameliorating symptoms associated with respiratory syncytial virus (RSV) infection utilizing said compositions. In particular, the present invention relates to methods for  
10 preventing, treating or ameliorating symptoms associated with RSV infection, said methods comprising administering to a human subject an effective amount of one or more antibodies or fragments thereof that immunospecifically bind to a RSV antigen, wherein a certain serum titer of said antibodies or antibody fragments is achieved in said human subject. The present invention also relates to detectable or diagnostic compositions comprising  
15 antibodies or fragments thereof that immunospecifically bind to a RSV antigen and methods for detecting or diagnosing RSV infection utilizing said compositions.

**2.     BACKGROUND OF THE INVENTION**

Respiratory syncytial virus (RSV) is the leading cause of serious lower respiratory  
20 tract disease in infants and children (Feigen et al., eds., 1987, *In: Textbook of Pediatric Infectious Diseases*, WB Saunders, Philadelphia at pages 1653-1675; New Vaccine Development, Establishing Priorities, Vol. 1, 1985, National Academy Press, Washington DC at pages 397-409; and Ruuskanen et al., 1993, *Curr. Probl. Pediatr.* 23:50-79). The yearly epidemic nature of RSV infection is evident worldwide, but the incidence and  
25 severity of RSV disease in a given season vary by region (Hall, C.B., 1993, *Contemp. Pediatr.* 10:92-110). In temperate regions of the northern hemisphere, it usually begins in late fall and ends in late spring. Primary RSV infection occurs most often in children from 6 weeks to 2 years of age and uncommonly in the first 4 weeks of life during nosocomial epidemics (Hall et al., 1979, *New Engl. J. Med.* 300:393-396). Children at increased risk  
30 from RSV infection include preterm infants (Hall et al., 1979, *New Engl. J. Med.* 300:393-396) and children with bronchopulmonary dysplasia (Groothuis et al., 1988, *Pediatrics* 82:199-203), congenital heart disease (MacDonald et al., *New Engl. J. Med.* 307:397-400), congenital or acquired immunodeficiency (Ogra et al., 1988, *Pediatr. Infect. Dis. J.* 7:246-249; and Pohl et al., 1992, *J. Infect. Dis.* 165:166-169), and cystic fibrosis (Abman et al.,  
35



1988, J. Pediatr. 113:826-830). The fatality rate in infants with heart or lung disease who are hospitalized with RSV infection is 3%-4% (Navas et al., 1992, J. Pediatr. 121:348-354).

RSV infects adults as well as infants and children. In healthy adults, RSV causes predominantly upper respiratory tract disease. It has recently become evident that some adults, especially the elderly, have symptomatic RSV infections more frequently than had been previously reported (Evans, A.S., eds., 1989, *Viral Infections of Humans. Epidemiology and Control*, 3<sup>rd</sup> ed., Plenum Medical Book, New York at pages 525-544). Several epidemics also have been reported among nursing home patients and institutionalized young adults (Falsey, A.R., 1991, *Infect. Control Hosp. Epidemiol.* 12:602-608; and Garvie et al., 1980, *Br. Med. J.* 281:1253-1254). Finally, RSV may cause serious disease in immunosuppressed persons, particularly bone marrow transplant patients (Hertz et al., 1989, *Medicine* 68:269-281).

Treatment options for established RSV disease are limited. Severe RSV disease of the lower respiratory tract often requires considerable supportive care, including administration of humidified oxygen and respiratory assistance (Fields et al., eds, 1990, *Fields Virology*, 2<sup>nd</sup> ed., Vol. 1, Raven Press, New York at pages 1045-1072). The only drug approved for treatment of infection is the antiviral agent ribavirin (American Academy of Pediatrics Committee on Infectious Diseases, 1993, *Pediatrics* 92:501-504). It has been shown to be effective in the treatment of RSV pneumonia and bronchiolitis, modifying the course of severe RSV disease in immunocompetent children (Smith et al., 1991, *New Engl. J. Med.* 325:24-29). However, ribavirin has had limited use because it requires prolonged aerosol administration and because of concerns about its potential risk to pregnant women who may be exposed to the drug during its administration in hospital settings.

While a vaccine might prevent RSV infection, no vaccine is yet licensed for this indication. A major obstacle to vaccine development is safety. A formalin-inactivated vaccine, though immunogenic, unexpectedly caused a higher and more severe incidence of lower respiratory tract disease due to RSV in immunized infants than in infants immunized with a similarly prepared trivalent parainfluenza vaccine (Kim et al., 1969, *Am. J. Epidemiol.* 89:422-434; and Kapikian et al., 1969, *Am. J. Epidemiol.* 89:405-421). Several candidate RSV vaccines have been abandoned and others are under development (Murphy et al., 1994, *Virus Res.* 32:13-36), but even if safety issues are resolved, vaccine efficacy must also be improved. A number of problems remain to be solved. Immunization would be required in the immediate neonatal period since the peak incidence of lower respiratory tract disease occurs at 2-5 months of age. The immaturity of the neonatal immune response together with high titers of maternally acquired RSV antibody may be expected to reduce

vaccine immunogenicity in the neonatal period (Murphy et al., 1988, *J. Virol.* 62:3907-3910; and Murphy et al., 1991, *Vaccine* 9:185-189). Finally, primary RSV infection and disease do not protect well against subsequent RSV disease (Henderson et al., 1979, *New Engl. J. Med.* 300:530-534).

5           Currently, the only approved approach to prophylaxis of RSV disease is passive immunization. Initial evidence suggesting a protective role for IgG was obtained from observations involving maternal antibody in ferrets (Prince, G.A., Ph.D. diss., University of California, Los Angeles, 1975) and humans (Lambrecht et al, 1976, *J. Infect. Dis.* 134:211-217; and Glezen et al., 1981, *J. Pediatr.* 98:708-715). Hemming et al. (Morell et al., eds.,  
10 1986, *Clinical Use of Intravenous Immunoglobulins*, Academic Press, London at pages 285-294) recognized the possible utility of RSV antibody in treatment or prevention of RSV infection during studies involving the pharmacokinetics of an intravenous immune globulin (IVIG) in newborns suspected of having neonatal sepsis. They noted that 1 infant, whose respiratory secretions yielded RSV, recovered rapidly after IVIG infusion. Subsequent  
15 analysis of the IVIG lot revealed an unusually high titer of RSV neutralizing antibody. This same group of investigators then examined the ability of hyperimmune serum or immune globulin, enriched for RSV neutralizing antibody, to protect cotton rats and primates against RSV infection (Prince et al., 1985, *Virus Res.* 3:193-206; Prince et al., 1990, *J. Virol.* 64:3091-3092; Hemming et al., 1985, *J. Infect. Dis.* 152:1083-1087; Prince et al., 1983,  
20 *Infect. Immun.* 42:81-87; and Prince et al., 1985, *J. Virol.* 55:517-520). Results of these studies suggested that RSV neutralizing antibody given prophylactically inhibited respiratory tract replication of RSV in cotton rats. When given therapeutically, RSV antibody reduced pulmonary viral replication both in cotton rats and in a nonhuman primate model. Furthermore, passive infusion of immune serum or immune globulin did not  
25 produce enhanced pulmonary pathology in cotton rats subsequently challenged with RSV.

          Recent clinical studies have demonstrated the ability of this passively administered RSV hyperimmune globulin (RSV IVIG) to protect at-risk children from severe lower respiratory infection by RSV (Groothuis et al., 1993, *New Engl. J. Med.* 329:1524-1530; and The PREVENT Study Group, 1997, *Pediatrics* 99:93-99). While this is a major  
30 advance in preventing RSV infection, this treatment poses certain limitations in its widespread use. First, RSV IVIG must be infused intravenously over several hours to achieve an effective dose. Second, the concentrations of active material in hyperimmune globulins are insufficient to treat adults at risk or most children with compromised cardiopulmonary function. Third, intravenous infusion necessitates monthly hospital visits  
35 during the RSV season. Finally, it may prove difficult to select sufficient donors to produce

a hyperimmune globulin for RSV to meet the demand for this product. Currently, only approximately 8% of normal donors have RSV neutralizing antibody titers high enough to qualify for the production of hyperimmune globulin.

One way to improve the specific activity of the immunoglobulin would be to  
5 develop one or more highly potent RSV neutralizing monoclonal antibodies (MAbs). Such MAbs should be human or humanized in order to retain favorable pharmacokinetics and to avoid generating a human anti-mouse antibody response, as repeat dosing would be required throughout the RSV season. Two glycoproteins, F and G, on the surface of RSV have been shown to be targets of neutralizing antibodies (Fields et al., 1990, *supra*; and Murphy et al.,  
10 1994, *supra*). These two proteins are also primarily responsible for viral recognition and entry into target cells; G protein binds to a specific cellular receptor and the F protein promotes fusion of the virus with the cell. The F protein is also expressed on the surface of infected cells and is responsible for subsequent fusion with other cells leading to syncytia formation. Thus, antibodies to the F protein may directly neutralize virus or block entry of  
15 the virus into the cell or prevent syncytia formation. Although antigenic and structural differences between A and B subtypes have been described for both the G and F proteins, the more significant antigenic differences reside on the G glycoprotein, where amino acid sequences are only 53% homologous and antigenic relatedness is 5% (Walsh et al., 1987, J. Infect. Dis. 155:1198-1204; and Johnson et al., 1987, Proc. Natl. Acad. Sci. USA 84:5625-  
20 5629). Conversely, antibodies raised to the F protein show a high degree of cross-reactivity among subtype A and B viruses. Beeler and Coelingh (1989, J. Virol. 7:2941-2950) conducted an extensive analysis of 18 different murine MAbs directed to the RSV F protein. Comparison of the biologic and biochemical properties of these MAbs resulted in the identification of three distinct antigenic sites (designated A, B, and C). Neutralization  
25 studies were performed against a panel of RSV strains isolated from 1956 to 1985 that demonstrated that epitopes within antigenic sites A and C are highly conserved, while the epitopes of antigenic site B are variable.

A humanized antibody directed to an epitope in the A antigenic site of the F protein of RSV, SYNAGIS®, is approved for intramuscular administration to pediatric patients for  
30 prevention of serious lower respiratory tract disease caused by RSV at recommended monthly doses of 15 mg/kg of body weight throughout the RSV season (November through April in the northern hemisphere). SYNAGIS® is a composite of human (95%) and murine (5%) antibody sequences. See, Johnson et al., 1997, J. Infect. Diseases 176:1215-1224 and U.S. Patent No. 5,824,307, the entire contents of which are incorporated herein by  
35 reference. The human heavy chain sequence was derived from the constant domains of

human IgG<sub>1</sub> and the variable framework regions of the VH genes or Cor (Press et al., 1970, Biochem. J. 117:641-660) and Cess (Takashi et al., 1984, Proc. Natl. Acad. Sci. USA 81:194-198). The human light chain sequence was derived from the constant domain of C $\kappa$  and the variable framework regions of the VL gene K104 with J $\kappa$ -4 (Bentley et al., 1980, Nature 288:5194-5198). The murine sequences derived from a murine monoclonal antibody, Mab 1129 (Beeler et al., 1989, J. Virology 63:2941-2950), in a process which involved the grafting of the murine complementarity determining regions into the human antibody frameworks.

Although SYNAGIS® has been successfully used for the prevention of RSV infection in pediatric patients, multiple intramuscular doses of 15 mg/kg of SYNAGIS™ is required to achieve a prophylactic effect. In pediatric patients less than 24 months of age, the mean half-life of SYNAGIS® has been shown to be 20 days and monthly intramuscular doses of 15 mg/kg have been shown to result in a mean  $\pm$  standard derivation 30 day serum titer of  $37 \pm 21$   $\mu$ g/ml after the first injection,  $57 \pm 41$   $\mu$ g/ml after the second injection,  $68 \pm 51$   $\mu$ g/ml after the third injection, and  $72 \pm 50$   $\mu$ g/ml after the fourth injection (The IMPact RSV Study Group, 1998, Pediatrics 102:531-537). Serum concentrations of greater than 30  $\mu$ g/ml have been shown to be necessary to reduce pulmonary RSV replication by 100 fold in the cotton rat model of RSV infection. However, the administration of multiple intramuscular doses of 15 mg/kg of antibody is inconvenient for the patient. Thus, a need exists for antibodies that immunospecifically bind to a RSV antigen, which are highly potent, have an improved pharmacokinetic profile, and thus have an overall improved therapeutic profile. Further, a need exists for antibodies that immunospecifically bind to a RSV antigen which require less frequent administration.

Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

### Summary of the Invention

According to a first embodiment of the invention there is provided an isolated antibody comprising:

- (a) a variable light (VL) chain comprising the amino acid sequence of SEQ ID NO:257;
- (b) a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (c) a variable heavy (VH) chain comprising the amino acid sequence of SEQ ID NO:256, and

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- (d) a VL chain comprising the amino acid sequence of SEQ ID NO:257;  
a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (e) a VH chain comprising the amino acid sequence of SEQ ID NO:256, and  
a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (f) a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- 10 (g) a VL chain or VL domain comprising a VL complementary determining  
region (CDR) 1 having the amino acid sequence of SEQ ID NO:39, a VL  
CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3  
having the amino acid sequence of SEQ ID NO:6;
- 15 (h) a VH chain or VH domain comprising a VH CDR1 having the amino acid  
sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of  
SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID  
NO:20; and  
a VL chain or VL domain comprising a VL CDR1 having the amino acid  
sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of  
SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID  
NO:6;
- 20 (i) a VH chain comprising the amino acid sequence of SEQ ID NO:256; and  
a VL chain or VL domain comprising a VL CDR1 comprising the amino acid  
sequence of SEQ ID NO:39, a VL CDR2 comprising the amino acid sequence  
of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ  
ID NO:6;
- 25 (j) a VH domain comprising the amino acid sequence of SEQ ID NO:48; and  
a VL chain or VL domain comprising a VL CDR1 having the amino acid  
sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of  
SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID  
NO:6;
- 30 (k) a VH chain or VH domain comprising a VH CDR1 having the amino acid  
sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of  
SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID  
NO:20; and  
a VL domain comprising the amino acid sequence of SEQ ID NO:76; or

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- (l) a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and  
 a VL chain comprising the amino acid sequence of SEQ ID NO:257.

According to a second embodiment of the invention, there is provided a composition comprising the antibody in accordance with the first embodiment of the present invention and a pharmacologically acceptable carrier.

According to a third embodiment of the invention there is provided an isolated nucleic acid molecule comprising a nucleotide sequence encoding:

- (a) a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- (b) a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (c) a VH chain comprising the amino acid sequence of SEQ ID NO:256, and  
 a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- (d) a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
 a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (e) a VH chain comprising the amino acid sequence of SEQ ID NO:256, and  
 a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (f) a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
 a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- (g) a VL chain or VL domain comprising a VL complementary determining region (CDR) 1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6;
- (h) a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and  
 a VL chain or VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6;
- (i) a VH chain comprising the amino acid sequence of SEQ ID NO:256; and  
 a VL chain or VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence

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of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6;

- (j) a VH domain comprising the amino acid sequence of SEQ ID NO:48; and a VL chain or VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6;
- (k) a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and
- (l) a VL domain comprising the amino acid sequence of SEQ ID NO:76; or a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and
- a VL chain comprising the amino acid sequence of SEQ ID NO:257.

According to a fourth embodiment of the invention, there is provided a vector comprising the nucleic acid molecule in accordance with the third embodiment of the present invention.

According to a fifth embodiment of the invention, there is provided a host cell genetically engineered to contain or express the nucleic acid molecule in accordance with the third embodiment of the present invention.

According to a sixth embodiment of the invention, there is provided a host cell comprising the vector in accordance with the fourth embodiment of the present invention.

According to a seventh embodiment of the invention, there is provided a method for producing an antibody, comprising culturing the host cell in accordance with the fifth or sixth embodiment of the present invention under conditions in which the nucleic acid molecule is expressed.

According to an eighth embodiment of the invention, there is provided a kit comprising the antibody in accordance with the first embodiment of the present invention, in a container, and instructions for use.

According to a ninth embodiment of the invention, there is provided a composition comprising the isolated nucleic acid in accordance with the third embodiment of the present invention and a pharmaceutically acceptable carrier.

According to a tenth embodiment of the invention, there is provided a kit comprising the isolated nucleic acid in accordance with the third embodiment of the present invention, in a container, and instructions for use.

5 According to an eleventh embodiment of the invention, there is provided a fusion protein comprising a non-IgG polypeptide recombinantly fused to the antibody in accordance with the first embodiment of the present invention.

According to a twelfth embodiment of the invention, there is provided a composition comprising the fusion protein in accordance with the eleventh embodiment of the present invention and a pharmaceutically acceptable carrier.

10 According to a thirteenth embodiment of the invention, there is provided a kit comprising the fusion protein in accordance with the eleventh embodiment of the present invention, in a container, and instructions for use.

According to a fourteenth embodiment of the invention, there is provided an antibody conjugate comprising a non-IgG polypeptide chemically conjugated to the antibody in accordance with the first embodiment of the present invention.

15 According to a fifteenth embodiment of the invention, there is provided a composition comprising the antibody conjugate in accordance with the fourteenth embodiment of the present invention and a pharmaceutically acceptable carrier.

20 According to a sixteenth embodiment of the invention, there is provided a kit comprising the antibody conjugate in accordance with the fourteenth embodiment of the present invention, in a container, and instructions for use.

According to a seventeenth embodiment of the invention, there is provided a method of producing the fusion protein in accordance with the eleventh embodiment of the present invention, comprising:

- 25 (a) transforming a host cell with a vector comprising a promoter operably linked to the nucleotide sequence encoding the fusion protein, and  
(b) culturing said host cell to produce the fusion protein.

According to an eighteenth embodiment of the invention, there is provided a method for detecting or diagnosing a RSV infection, comprising:

- 30 (a) contacting a cell or tissue sample of a subject with the antibody in accordance with the first embodiment of the present invention; and  
(b) detecting the level of a RSVF antigen in the cell or the tissue sample;

wherein an increase in the level of RSV antigen the cell or tissue sample of the subject compared to a control level of RSV antigen in a cell or tissue sample not infected with RSV is indicative of a RSV infection.

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According to a nineteenth embodiment of the invention, there is provided a method for detecting or diagnosing a RSV infection, comprising:

(a) contacting a cell or tissue sample of a subject with the fusion protein in accordance with the eleventh embodiment of the present invention; and

5 (b) detecting the level of a RSVF antigen in the cell or the tissue sample;

wherein an increase in the level of RSV antigen the cell or tissue sample of the subject compared to a control level of RSV antigen in a cell or tissue sample not infected with RSV is indicative of a RSV infection.

According to a twentieth embodiment of the invention, there is provided a method  
10 for detecting or diagnosing a RSV infection, comprising:

(a) contacting a cell or tissue sample of a subject with the antibody conjugate in accordance with the thirteenth or fourteenth embodiment of the present invention ; and

(b) detecting the level of a RSVF antigen in the cell or the tissue sample;

15 wherein an increase in the level of RSV antigen the cell or tissue sample of the subject compared to a control level of RSV antigen in a cell or tissue sample not infected with RSV is indicative of a RSV infection.

According to a twenty-first embodiment of the invention, there is provided a sustained release formulation comprising the antibody in accordance with the first embodiment of the present invention.

20 According to a twenty-second embodiment of the invention, there is provided a composition comprising the antibody in accordance with the first embodiment of the present invention formulated for pulmonary delivery.

According to a twenty-third embodiment of the invention, there is provided a composition comprising the antibody in accordance with the first embodiment of the  
25 present invention formulated for intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, or intranasal delivery.

According to a twenty-fourth embodiment of the invention, there is provided a method for preventing a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the antibody in accordance with the  
30 first embodiment of the present invention.

According to a twenty-fifth embodiment of the invention, there is provided a method for preventing a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the composition in accordance with the second embodiment of the present invention.

According to a twenty-sixth embodiment of the invention, there is provided a method of preventing a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the sustained release formulation in accordance with the twenty-first embodiment of the present invention.

5 According to a twenty-seventh embodiment of the invention, there is provided a method of preventing a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the composition in accordance with the twenty-third embodiment of the present invention.

10 According to a twenty-eighth embodiment of the invention, there is provided a method of preventing a RSV infection or a symptom thereof in a mammal, comprising administering to the lungs of said mammal an effective amount of the composition in accordance with the twenty-second embodiment of the present invention.

15 According to a twenty-ninth embodiment of the invention, there is provided a method for treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the antibody in accordance with the first embodiment of the present invention.

20 According to a thirtieth embodiment of the invention, there is provided a method for treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the composition in accordance with the second embodiment of the present invention.

25 According to a thirty-first embodiment of the invention, there is provided a method of treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the sustained release formulation in accordance with the twenty-first embodiment of the present invention.

30 According to a thirty-second embodiment of the invention, there is provided a method of treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the composition in accordance with the twenty-third embodiment of the present invention.

35 According to a thirty-third embodiment of the invention, there is provided a method of treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to the lungs of said mammal an effective amount of the composition in accordance with the twenty-second embodiment of the present invention.

According to a thirty-fourth embodiment of the invention, there is provided a method for reducing RSV titers in a mammal, comprising administering to said mammal

an effective amount of the antibody in accordance with the first embodiment of the present invention.

According to a thirty-fifth embodiment of the invention, there is provided a method for reducing RSV titers in a mammal, comprising administering to said mammal an effective amount of the composition in accordance with the second embodiment of the present invention.

According to a thirty-sixth embodiment of the invention, there is provided a method for reducing RSV titers in a mammal, comprising administering to said mammal an effective amount of the sustained release formulation in accordance with the twenty-first embodiment of the present invention.

According to a thirty-seventh embodiment of the invention, there is provided a method for reducing RSV titers in a mammal, comprising administering to said mammal an effective amount of the composition in accordance with the twenty-third embodiment of the present invention.

The present invention is based, in part, on the development of methods for achieving or inducing a prophylactically or therapeutically effective serum titer of an antibody or fragment thereof that immunospecifically binds to a respiratory syncytial virus (RSV) antigen in a mammal by passive immunization with such an antibody or fragment thereof, which methods require lower dosages and/or less frequent administration than previously known methods. The present invention is also based, in part, on the identification of antibodies with higher affinities for a RSV antigen which result in increased efficacy for prophylactic or therapeutic uses such that lower serum titers are prophylactically or

therapeutically effective, thereby permitting administration of lower dosages and/or reduced frequency of administration.

The present invention provides methods of preventing, neutralizing, treating and ameliorating one or more symptoms associated with RSV infection in a subject comprising  
5 administering to said subject one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with high affinity and/or high avidity. Because a lower serum titer of such antibodies or antibody fragments is therapeutically or prophylactically effective than the effective serum titer of known  
10 antibodies, lower doses of said antibodies or antibody fragments can be used to achieve a serum titer effective for the prevention, neutralization, treatment and the amelioration of symptoms associated with a RSV infection. The use of lower doses of antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens reduces the likelihood of adverse effects. Further, the high affinity and/or high avidity of the antibodies of the invention or fragments thereof enable less frequent administration of said antibodies  
15 or antibody fragments than previously thought to be necessary for the prevention, neutralization, treatment or the amelioration of symptoms associated with a RSV infection.

The present invention also provides antibodies which immunospecifically bind to one or more RSV antigens and have increased *in vivo* half-lives relative to known antibodies such as, *e.g.*, SYNAGIS®. In particular, the present invention encompasses  
20 antibodies which immunospecifically bind to one or more RSV antigens and have increased *in vivo* half-lives relative to known antibodies (*e.g.*, SYNAGIS®), said increased half-lives resulting from one or more modifications (*e.g.*, substitutions, deletions, or insertions) in amino acid residues identified to be involved in the interaction of the Fc domain of said antibodies and the FcRn receptor. The present invention also encompasses pegylated  
25 antibodies and fragments thereof which immunospecifically bind to one or more RSV antigens and have increased *in vivo* half-lives relative to known antibodies such as, *e.g.*, SYNAGIS®. The increased *in vivo* half-lives of antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens reduce the dosage and/or frequency of administration of said antibodies or fragments thereof to a subject.

30 The invention encompasses sustained release formulations for the administration of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens to a subject. The sustained release formulations reduce the dosage and/or frequency of administration of said antibodies or antibody fragments to a subject. Further, the sustained release formulations may be administered to maintain a therapeutically or

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prophylactically effective serum titer which does not exceed a certain maximum serum titer for a certain period of time.

The present invention encompasses methods of delivering one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens directly to the site of RSV infection. In particular, the invention encompasses pulmonary delivery of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens. The improved methods of delivering of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens reduce the dosage and/or frequency of administration of said antibodies or antibody fragments to a subject.

The present invention provides antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and have an association rate constant or  $k_{on}$  rate (antibody (Ab) + antigen (Ag)  $\xrightarrow{k_{on}}$  Ab-Ag) of at least  $10^5 M^{-1}s^{-1}$ , at least  $5 \times 10^5 M^{-1}s^{-1}$ , at least  $10^6 M^{-1}s^{-1}$ , at least  $5 \times 10^6 M^{-1}s^{-1}$ , at least  $10^7 M^{-1}s^{-1}$ , at least  $5 \times 10^7 M^{-1}s^{-1}$ , or at least  $10^8 M^{-1}s^{-1}$ . In particular, the present invention provides compositions for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection, said compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and have an a  $k_{on}$  rate of at least  $10^5 M^{-1}s^{-1}$ , at least  $5 \times 10^5 M^{-1}s^{-1}$ , at least  $10^6 M^{-1}s^{-1}$ , at least  $5 \times 10^6 M^{-1}s^{-1}$ , at least  $10^7 M^{-1}s^{-1}$ , at least  $5 \times 10^7 M^{-1}s^{-1}$ , or at least  $10^8 M^{-1}s^{-1}$ .

The present invention provides antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and have a  $k_{off}$  rate (antibody (Ab) + antigen (Ag)  $\xleftarrow{k_{off}}$  Ab-Ag) of less than  $10^{-1} s^{-1}$ , less than  $5 \times 10^{-1} s^{-1}$ , less than  $10^{-2} s^{-1}$ , less than  $5 \times 10^{-2} s^{-1}$ , less than  $10^{-3} s^{-1}$ , less than  $5 \times 10^{-3} s^{-1}$ , less than  $10^{-4} s^{-1}$ , less than  $5 \times 10^{-4} s^{-1}$ , less than  $10^{-5} s^{-1}$ , less than  $5 \times 10^{-5} s^{-1}$ , less than  $10^{-6} s^{-1}$ , less than  $5 \times 10^{-6} s^{-1}$ , less than  $10^{-7} s^{-1}$ , less than  $5 \times 10^{-7} s^{-1}$ , less than  $10^{-8} s^{-1}$ , less than  $5 \times 10^{-8} s^{-1}$ , less than  $10^{-9} s^{-1}$ , less than  $5 \times 10^{-9} s^{-1}$ , or less than  $10^{-10} s^{-1}$ . In particular, the present invention provides compositions for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection, said compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and have a  $k_{off}$  rate of less than  $10^{-1} s^{-1}$ , less than  $5 \times 10^{-1} s^{-1}$ , less than  $10^{-2} s^{-1}$ , less than  $5 \times 10^{-2} s^{-1}$ , less than  $10^{-3} s^{-1}$ , less than  $5 \times 10^{-3} s^{-1}$ , less than  $10^{-4} s^{-1}$ , less than  $5 \times 10^{-4} s^{-1}$ , less than  $10^{-5} s^{-1}$ , less than  $5 \times 10^{-5} s^{-1}$ , less than  $10^{-6} s^{-1}$ , less than  $5 \times 10^{-6} s^{-1}$ , less than  $10^{-7} s^{-1}$ , less than  $5 \times 10^{-7} s^{-1}$ , less than  $10^{-8} s^{-1}$ , less than  $5 \times 10^{-8} s^{-1}$ , less than  $10^{-9} s^{-1}$ , or less than  $10^{-10} s^{-1}$ .

The present invention also provides antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and have an affinity constant or  $K_a$

( $k_{on}/k_{off}$ ) of at least  $10^2 M^{-1}$ , at least  $5 \times 10^2 M^{-1}$ , at least  $10^3 M^{-1}$ , at least  $5 \times 10^3 M^{-1}$ , at least  $10^4 M^{-1}$ , at least  $5 \times 10^4 M^{-1}$ , at least  $10^5 M^{-1}$ , at least  $5 \times 10^5 M^{-1}$ , at least  $10^6 M^{-1}$ , at least  $5 \times 10^6 M^{-1}$ , at least  $10^7 M^{-1}$ , at least  $5 \times 10^7 M^{-1}$ , at least  $10^8 M^{-1}$ , at least  $5 \times 10^8 M^{-1}$ , at least  $10^9 M^{-1}$ , at least  $5 \times 10^9 M^{-1}$ , at least  $10^{10} M^{-1}$ , at least  $5 \times 10^{10} M^{-1}$ , at least  $10^{11} M^{-1}$ , at least  $5 \times 10^{11} M^{-1}$ , at least  $10^{12} M^{-1}$ , at least  $5 \times 10^{12} M^{-1}$ , at least  $10^{13} M^{-1}$ , at least  $5 \times 10^{13} M^{-1}$ , at least  $10^{14} M^{-1}$ , at least  $5 \times 10^{14} M^{-1}$ , at least  $10^{15} M^{-1}$ , or at least  $5 \times 10^{15} M^{-1}$ . In particular, the present invention provides compositions for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection, said compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and have a  $K_a$  of at least  $10^2 M^{-1}$ , at least  $5 \times 10^2 M^{-1}$ , at least  $10^3 M^{-1}$ , at least  $5 \times 10^3 M^{-1}$ , at least  $10^4 M^{-1}$ , at least  $5 \times 10^4 M^{-1}$ , at least  $10^5 M^{-1}$ , at least  $5 \times 10^5 M^{-1}$ , at least  $10^6 M^{-1}$ , at least  $5 \times 10^6 M^{-1}$ , at least  $10^7 M^{-1}$ , at least  $5 \times 10^7 M^{-1}$ , at least  $10^8 M^{-1}$ , at least  $5 \times 10^8 M^{-1}$ , at least  $10^9 M^{-1}$ , at least  $5 \times 10^9 M^{-1}$ , at least  $10^{10} M^{-1}$ , at least  $5 \times 10^{10} M^{-1}$ , at least  $10^{11} M^{-1}$ , at least  $5 \times 10^{11} M^{-1}$ , at least  $10^{12} M^{-1}$ , at least  $5 \times 10^{12} M^{-1}$ , at least  $10^{13} M^{-1}$ , at least  $5 \times 10^{13} M^{-1}$ , at least  $10^{14} M^{-1}$ , at least  $5 \times 10^{14} M^{-1}$ , at least  $10^{15} M^{-1}$ , or at least  $5 \times 10^{15} M^{-1}$ .

The present invention provides antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and have a median effective concentration ( $EC_{50}$ ) of less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM, less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM, in an *in vitro* microneutralization assay. In particular, the present invention provides compositions for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection, said compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and have an  $EC_{50}$  of less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM, less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM, in an *in vitro* microneutralization assay.

The present invention also provides antibodies or fragments thereof comprising a VH domain having the amino acid sequence of any VH domain listed in Table 2 and compositions comprising said antibodies or antibody fragments for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection. The present invention also provides antibodies or fragments thereof comprising one or more VH complementarity determining regions (CDRs) having the amino acid sequence of one or more VH CDRs listed in Table 2 and/or Table 3 and compositions comprising said

antibodies or antibody fragments for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection. The present invention also provides antibodies or fragments thereof comprising a VL domain having the amino acid sequence of any VL domain listed in Table 2. The present invention also provides antibodies or  
5 fragments thereof comprising one or more VL CDRs having the amino acid sequence of one or more VL CDRs listed in Table 2 and/or Table 3 and compositions comprising said antibodies or antibody fragments for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection. The present invention further provides antibodies comprising a VH domain and a VL domain having the amino acid sequence of  
10 any VH domain and VL domain listed in Table 2 and compositions comprising said antibodies or antibody fragments for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection. The present invention further provides antibodies comprising one or more VH CDRs and one or more VL CDRs having the amino acid sequence of one or more VH CDRs and one or more VL CDRs listed in Table 2 and/or  
15 3 and compositions comprising said antibodies or antibody fragments for use in the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection. In the above embodiments, preferably the antibody binds immunospecifically to a RSV antigen.

The present invention also encompasses methods for achieving a serum titer of at  
20 least 40  $\mu\text{g/ml}$  of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens in a mammal, preferably a primate and most preferably a human. In particular, the present invention provides methods for achieving a serum titer of at least 40  $\mu\text{g/ml}$  (preferably at least 75  $\mu\text{g/ml}$ , more preferably at least 100  $\mu\text{g/ml}$ , and most preferably at least 150  $\mu\text{g/ml}$ ) of an antibody or fragment thereof that immunospecifically  
25 binds to a RSV antigen in a non-primate mammal, comprising administering a dose of less than 2.5 mg/kg (preferably 1.5 mg/kg or less) of the antibody or antibody fragment to the non-primate mammal and measuring the serum titer of the antibody or antibody fragment at least 1 day after administering the dose to the non-primate mammal. The present invention also provides methods for achieving a serum titer of at least 150  $\mu\text{g/ml}$  (preferably at least  
30 200  $\mu\text{g/ml}$ ) of an antibody or fragment thereof that immunospecifically binds to a RSV antigen in a non-primate mammal, comprising administering a dose of approximately 5 mg/kg of the antibody or antibody fragment to the non-primate mammal and measuring the serum titer of the antibody or antibody fragment at least 1 day after the administration of the dose to the non-primate mammal.

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The present invention also provides methods for achieving a serum titer of at least 40 µg/ml of an antibody or fragment thereof that immunospecifically binds to a RSV antigen in a primate, comprising administering a first dose of 10 mg/kg (preferably 5 mg/kg or less and more preferably 1.5 mg/kg or less) of the antibody or antibody fragment to the primate and measuring the serum titer of the antibody or antibody fragment 20 days (preferably 25, 30, 35 or 40 days) after administering the first dose to the primate and prior to the administration of any subsequent dose. The present invention also provides methods for achieving a serum titer of at least 75 µg/ml (preferably at least 100 µg/ml, at least 150 µg/ml, or at least 200 µg/ml) of an antibody or fragment thereof that immunospecifically binds to a RSV antigen in a primate, comprising administering a first dose of approximately 15 mg/kg of the antibody or antibody fragment to the primate and measuring the serum titer of the antibody or antibody fragment 20 days (preferably 25, 30, 35 or 40 days) after administering the first dose to the primate but prior to any subsequent dose.

The present invention also provides methods for preventing, treating, or ameliorating one or more symptoms associated with a RSV infection in a human subject, said methods comprising administering to said human subject at least a first dose of approximately 15 mg/kg of an antibody or fragment thereof that immunospecifically binds to a RSV antigen so that said human subject has a serum antibody titer of at least 75 µg/ml, preferably at least 100 µg/ml, at least 150 µg/ml, or at least 200 µg/ml 30 days after the administration of the first dose of the antibody or antibody fragment and prior to the administration of a subsequent dose. The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a human subject, said methods comprising administering to said human subject at least a first dose of less than 15 mg/kg (preferably 10 mg/kg or less, more preferably 5 mg/kg or less, and most preferably 1.5 mg/kg or less) of an antibody or fragment thereof that immunospecifically binds to a RSV antigen so that said human subject has a serum antibody titer of at least 75 µg/ml, preferably at least 100 µg/ml, at least 150 µg/ml, or at least 200 µg/ml 30 days after the administration of the first dose of the antibody or antibody fragment and prior to the administration of a subsequent dose. The present invention further provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a human subject, said methods comprising administering to said human subject a first dose of an antibody or fragment thereof that immunospecifically binds to a RSV antigen such that a prophylactically or therapeutically effective serum titer of less than 10 µg/ml is achieved no more than 30 days after administering the antibody or antibody fragment.



The present invention provides methods for achieving a therapeutically or prophylactically effective serum titer in a mammal, said methods comprising administering to said mammal an antibody or fragment thereof which immunospecifically binds to a RSV antigen and which has a  $k_{on}$  rate (antibody (Ab) + antigen (Ag)  $\xrightarrow{k_{on}}$  Ab-Ag) of at least  $2.5 \times 10^5 M^{-1} s^{-1}$ , preferably at least  $3 \times 10^5 M^{-1} s^{-1}$ , at least  $5 \times 10^5 M^{-1} s^{-1}$ , at least  $10^6 M^{-1} s^{-1}$ , at least  $5 \times 10^6 M^{-1} s^{-1}$ , at least  $10^7 M^{-1} s^{-1}$ , at least  $5 \times 10^7 M^{-1} s^{-1}$  or at least  $10^8 M^{-1} s^{-1}$ . In particular, the present invention provides methods for achieving a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30  $\mu g/ml$  (and is preferably at least 2  $\mu g/ml$ , more preferably at least 4  $\mu g/ml$ , and most preferably at least 6  $\mu g/ml$ ) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period, comprising administering to a mammal an antibody or fragment thereof which immunospecifically binds to a RSV antigen and which has a  $k_{on}$  rate of at least  $2.5 \times 10^5 M^{-1} s^{-1}$ , preferably at least  $3 \times 10^5 M^{-1} s^{-1}$ , at least  $5 \times 10^5 M^{-1} s^{-1}$ , at least  $10^6 M^{-1} s^{-1}$ , at least  $5 \times 10^6 M^{-1} s^{-1}$ , at least  $10^7 M^{-1} s^{-1}$ , at least  $5 \times 10^7 M^{-1} s^{-1}$ , or at least  $10^8 M^{-1} s^{-1}$ . Preferably, the antibody or antibody fragment has a higher  $k_{on}$  rate than SYNAGIS®.

The present invention also provides methods of neutralizing RSV using an antibody or fragment thereof which immunospecifically bind to a RSV antigen and which has a  $k_{on}$  rate of at least  $2.5 \times 10^5 M^{-1} s^{-1}$ , preferably at least  $3 \times 10^5 M^{-1} s^{-1}$ , at least  $5 \times 10^5 M^{-1} s^{-1}$ , at least  $10^6 M^{-1} s^{-1}$ , at least  $5 \times 10^6 M^{-1} s^{-1}$ , at least  $10^7 M^{-1} s^{-1}$ , at least  $5 \times 10^7 M^{-1} s^{-1}$ , or at least  $10^8 M^{-1} s^{-1}$  to achieve a prophylactically or therapeutically effective serum titer, wherein said effective serum titer is less than 30  $\mu g/ml$  (and is preferably at least 2  $\mu g/ml$ , more preferably at least 4  $\mu g/ml$ , and most preferably at least 6  $\mu g/ml$ ) 20, 25, 30, or 35 days after administration without any other dosage administration. Preferably, the antibody or antibody fragment has a higher  $k_{on}$  rate than SYNAGIS®.

The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, said methods comprising administering to said mammal, a dose of less than 15 mg/kg (preferably 5 mg/kg or less, more preferably 3 mg/kg or less, and most preferably 1.5 mg/kg or less) of an antibody or fragment thereof which immunospecifically binds to a RSV antigen and has a  $k_{on}$  rate of at least  $2.5 \times 10^5 M^{-1} s^{-1}$ , preferably at least  $3 \times 10^5 M^{-1} s^{-1}$ , at least  $5 \times 10^5 M^{-1} s^{-1}$ , at least  $10^6 M^{-1} s^{-1}$ , at least  $5 \times 10^6 M^{-1} s^{-1}$ , at least  $10^7 M^{-1} s^{-1}$ , at least  $5 \times 10^7 M^{-1} s^{-1}$ , or at least  $10^8 M^{-1} s^{-1}$ . Preferably, the antibody or antibody fragment has a higher  $k_{on}$  rate for the RSV F glycoprotein than SYNAGIS®.

The present invention also provides methods for achieving a therapeutically or prophylactically effective serum titer in a mammal, said methods comprising administering to said mammal an antibody or fragment thereof which immunospecifically binds to a RSV antigen and which has a  $K_{\text{off}}$  rate (antibody (Ab) + antigen (Ag)  $\xrightleftharpoons{K_{\text{off}}} \text{Ab-Ag}$ ) of less than  $6.5 \times 10^{-4} \text{ sec}^{-1}$ , less than  $5 \times 10^{-4} \text{ sec}^{-1}$ , less than  $3 \times 10^{-4} \text{ sec}^{-1}$ , less than  $2 \times 10^{-4} \text{ sec}^{-1}$ , less than  $1 \times 10^{-4} \text{ sec}^{-1}$ , or less than  $5 \times 10^{-3} \text{ sec}^{-1}$ . In particular, the present invention provides methods for achieving a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than  $30 \mu\text{g/ml}$  (and is preferably at least  $2 \mu\text{g/ml}$ , more preferably at least  $4 \mu\text{g/ml}$ , and most preferably at least  $6 \mu\text{g/ml}$ ) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period, comprising administering to a mammal an antibody or fragment thereof which immunospecifically binds to a RSV antigen and which has a  $K_{\text{off}}$  rate of less than  $6.5 \times 10^{-4} \text{ sec}^{-1}$ , less than  $5 \times 10^{-4} \text{ sec}^{-1}$ , less than  $3 \times 10^{-4} \text{ sec}^{-1}$ , less than  $2 \times 10^{-4} \text{ sec}^{-1}$ , less than  $1 \times 10^{-4} \text{ sec}^{-1}$ , or less than  $5 \times 10^{-3} \text{ sec}^{-1}$ . Preferably, the antibody or fragment thereof has a lower  $K_{\text{off}}$  rate than SYNAGIS®.

The present invention also provides methods of neutralizing RSV using an antibody or antibody fragment thereof which immunospecifically binds to a RSV antigen and which has a  $K_{\text{off}}$  rate of less than  $6.5 \times 10^{-4} \text{ sec}^{-1}$ , less than  $5 \times 10^{-4} \text{ sec}^{-1}$ , less than  $3 \times 10^{-4} \text{ sec}^{-1}$ , less than  $2 \times 10^{-4} \text{ sec}^{-1}$ , less than  $1 \times 10^{-4} \text{ sec}^{-1}$ , or less than  $5 \times 10^{-3} \text{ sec}^{-1}$  to achieve a prophylactically or therapeutically effective serum titer, wherein said effective serum titer is less than  $30 \mu\text{g/ml}$  (and is preferably at least  $2 \mu\text{g/ml}$ , more preferably at least  $4 \mu\text{g/ml}$ , and most preferably at least  $6 \mu\text{g/ml}$ ) 20, 25, 30, or 35 days after administration without any other dosage administration. Preferably, the antibody or antibody fragment has a lower  $K_{\text{off}}$  than SYNAGIS®.

The present invention also provides methods for preventing, treating, or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, said methods comprising administering to a said mammal a dose of less than  $15 \text{ mg/kg}$  (preferably  $5 \text{ mg/kg}$  or less, more preferably  $3 \text{ mg/kg}$  or less, and most preferably  $1.5 \text{ mg/kg}$  or less) of an antibody or a fragment thereof which immunospecifically binds to a RSV antigen and which has a  $K_{\text{off}}$  rate of less than  $6.5 \times 10^{-4} \text{ sec}^{-1}$ , less than  $5 \times 10^{-4} \text{ sec}^{-1}$ , less than  $3 \times 10^{-4} \text{ sec}^{-1}$ , less than  $2 \times 10^{-4} \text{ sec}^{-1}$ , less than  $1 \times 10^{-4} \text{ sec}^{-1}$ , or less than  $5 \times 10^{-3} \text{ sec}^{-1}$ . Preferably, the antibody or antibody fragment has a lower  $K_{\text{off}}$  rate than SYNAGIS®.

The present invention also provides methods for achieving a therapeutically or prophylactically effective serum titer in a mammal, said methods comprising administering

to said mammal an antibody or fragment thereof which immunospecifically binds to a RSV antigen and which has an  $EC_{50}$  of less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM, less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM, in an *in vitro* microneutralization assay. In particular, the present invention provides methods for achieving a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30  $\mu\text{g/ml}$  (and is preferably at least 2  $\mu\text{g/ml}$ , more preferably at least 4  $\mu\text{g/ml}$ , and most preferably at least 6  $\mu\text{g/ml}$ ) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period, comprising administering to a mammal an antibody or fragment thereof which immunospecifically binds to a RSV antigen and which has an  $EC_{50}$  of less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM, less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM, in an *in vitro* microneutralization assay. Preferably, the antibody or antibody fragment has a lower  $EC_{50}$  than SYNAGIS®.

The present invention also provides methods of neutralizing RSV using an antibody or fragment thereof which immunospecifically binds to a RSV antigen and which has an  $EC_{50}$  of less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM, less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM, in an *in vitro* microneutralization assay to achieve a prophylactically or therapeutically effective serum titer, wherein said effective serum titer is less than 30  $\mu\text{g/ml}$  (and is preferably at least 2  $\mu\text{g/ml}$ , more preferably at least 4  $\mu\text{g/ml}$ , and most preferably at least 6  $\mu\text{g/ml}$ ) 20, 25, 30, or 35 days after administration without any other dosage administration. Preferably, the antibody or antibody fragment has a lower  $EC_{50}$  than SYNAGIS®.

The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, said methods comprising administering to said mammal a dose of less than 15 mg/kg (preferably 5 mg/kg or less, more preferably 3 mg/kg or less, and most preferably 1.5 mg/kg or less) of an antibody or a fragment thereof which immunospecifically binds to a RSV antigen and which has an  $EC_{50}$  of less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM, less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM, in an *in vitro* microneutralization assay. Preferably, the antibody or antibody fragment has a lower  $EC_{50}$  than SYNAGIS®.

The present invention provides methods for achieving a therapeutically or prophylactically effective serum titer in a mammal, said methods comprising administering to said mammal an antibody or fragment thereof which immunospecifically binds to a RSV antigen and which has an affinity constant ( $K_a$ ) for a RSV antigen of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$ . In particular, the present invention also provides methods for achieving a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than  $30 \mu\text{g/ml}$  (and is preferably at least  $2 \mu\text{g/ml}$ , more preferably at least  $4 \mu\text{g/ml}$ , and most preferably at least  $6 \mu\text{g/ml}$ ) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period, comprising administering to a mammal an antibody or fragment thereof that has an affinity constant ( $K_a$ ) for a RSV antigen of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$ . Preferably, the antibody or antibody fragment has a higher affinity for a RSV F glycoprotein than SYNAGIS®.

The present invention also provides methods of achieving a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than  $30 \mu\text{g/ml}$  (and is preferably at least  $2 \mu\text{g/ml}$ , more preferably at least  $4 \mu\text{g/ml}$ , and most preferably at least  $6 \mu\text{g/ml}$ ) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period, comprising administering to a mammal an antibody or fragment thereof which immunospecifically binds to a RSV antigen with a higher avidity than known antibodies such as, *e.g.*, SYNAGIS®.

The present invention also provides methods of neutralizing RSV using an antibody or fragment thereof that has an affinity constant ( $K_a$ ) for a RSV antigen of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$  to achieve a prophylactically or therapeutically effective serum titer, wherein said effective serum titer is less than  $30 \mu\text{g/ml}$  (and is at least  $2 \mu\text{g/ml}$  and more preferably at least  $6 \mu\text{g/ml}$ ) 20, 25, 30, or 35 days after administration

without any other dosage administration. Preferably, the antibody or antibody fragment has a higher affinity for the RSV F glycoprotein than SYNAGIS®. The present invention also provides methods of neutralizing RSV using an antibody or fragment thereof that has a higher avidity than known antibodies such as, *e.g.*, SYNAGIS®.

- 5       The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, said methods comprising administering to said mammal a dose of less than 15 mg/kg (preferably 5 mg/kg or less, more preferably 3 mg/kg or less, and most preferably 1.5 mg/kg or less) of an antibody or fragment thereof that has an affinity constant ( $K_a$ ) for a RSV
- 10 antigen of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$ . Preferably, the antibody or antibody fragment has a higher affinity for the RSV F glycoprotein than SYNAGIS®.
- 15 The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, said methods comprising administering to said mammal a first dose of less than 15 mg/kg (preferably 5 mg/kg or less, more preferably 3 mg/kg or less, and most preferably 1.5 mg/kg or less) of an antibody or fragment thereof that has a higher avidity than known antibodies
- 20 such as, *e.g.*, SYNAGIS®.

- The present invention encompasses methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, comprising administering to said mammal a first dose of a prophylactically or therapeutically effective amount of one or more antibodies or fragments thereof that
- 25 immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS®, wherein said effective amount is less than 15 mg/kg (preferably 5 mg/kg or less, more preferably 3 mg/kg or less, and most preferably 1.5 mg/kg or less) of said antibodies or antibody fragments which dose results in a serum titer of less than 30  $\mu\text{g/ml}$  (which is preferably at least 2  $\mu\text{g/ml}$ , more preferably at
- 30 least 4  $\mu\text{g/ml}$ , and most preferably at least 6  $\mu\text{g/ml}$ ) at least 20 days (preferably at least 25, at least 30, or at least 35 days) after the administration of the first dose and prior to the administration of a subsequent dose. In particular, the present invention provides methods for preventing, treating, or ameliorating one or more symptoms associated with a RSV infection in a human subject, comprising administering to said human subject a first dose of
- 35 less than 5 mg/kg (preferably 3 mg/kg or less, and most preferably 1.5 mg/kg) of an

antibody or fragment thereof that immunospecifically binds to a RSV antigen with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$ ) so that said human subject has a serum antibody titer of less than 30 µg/ml (which is preferably at least 2 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) at least 20 days (preferably at least 25, at least 30, or at least 35 days) after the administration of the first dose of the antibody or antibody fragment and prior to the administration of a subsequent dose.

10 The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising a VH domain having an amino acid sequence of any VH domain listed in Table 2 to achieve a therapeutically or prophylactically effective serum  
15 titer, wherein said effective serum titer is less than 30 µg/ml (and is preferably at least 2 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period. The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a  
20 mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising one or more VH complementarity determining regions (CDRs) having the amino acid sequence of one or more VH CDRs listed in Table 2 and/or Table 3 to achieve a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30 µg/ml (and is preferably at  
25 least 2 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period. Preferably, said antibodies or antibody fragments immunospecifically bind to a RSV antigen.

The present invention also provides methods for preventing, treating or ameliorating  
30 one or more symptoms associated with a RSV infection in a mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising a VL domain having the amino acid sequence of any VL domain listed in Table 2 to achieve a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30 µg/ml (and is preferably at least 2  
35 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) after a certain

number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period. The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising one or more VL CDRs having the amino acid sequence of one or more VL CDRs listed in Table 2 and/or Table 3 to achieve a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30  $\mu\text{g/ml}$  (and is preferably at least 2  $\mu\text{g/ml}$ , more preferably at least 4  $\mu\text{g/ml}$ , and most preferably at least 6  $\mu\text{g/ml}$ ) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period. Preferably, said antibodies or antibody fragments immunospecifically bind to a RSV antigen.

The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising a VH domain and a VL domain having the amino acid sequence of any VH domain and VL domain listed in Table 2 to achieve a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30  $\mu\text{g/ml}$  (and is preferably at least 2  $\mu\text{g/ml}$ , more preferably at least 4  $\mu\text{g/ml}$ , and most preferably at least 6  $\mu\text{g/ml}$ ) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period. The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising one or more VH CDRs and one or more VL CDRs having the amino acid sequence of one or more VH CDRs and one or more VL CDRs listed in Table 2 and/or 3 to achieve a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30  $\mu\text{g/ml}$  (and is preferably at least 2  $\mu\text{g/ml}$ , more preferably at least 4  $\mu\text{g/ml}$ , and most preferably at least 6  $\mu\text{g/ml}$ ) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period. Preferably, said antibodies or antibody fragments immunospecifically bind to a RSV antigen.

In a specific embodiment, the present invention provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising a VH domain having an amino acid sequence of SEQ ID NO:7, 9, 17, 24, 28, 33, 36, 40, 44, 48, 51, 67, or 78 and/or a VL

domain having an amino acid sequence of SEQ ID NO:8, 11, 13, 21, 26, 30, 34, 38, 42, 46, 49, 52, 54, 56, 58, 60, 62, 64, 65, 68, 70, 71, 74 or 76 to achieve a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30 µg/ml (and is preferably at least 2 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period. In a preferred embodiment, the present invention provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising a VH domain having an amino acid sequence of SEQ ID NO:9, 17, 24, 28, 33, 36, 40, 44, 48, 51, 55, 67 or 78 and/or a VL domain having an amino acid sequence of SEQ ID NO:13, 21, 26, 30, 34, 38, 42, 46, 49, 52, 54, 56, 58, 60, 62, 64, 65, 68, 70, 71, 74 or 76 to achieve a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30 µg/ml (and is preferably at least 2 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period. In another embodiment, the present invention provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, said methods comprising administering to said mammal a first dose of one or more antibodies or fragments thereof comprising a VH CDR3 having an amino acid sequence of SEQ ID NO:3, 12, 20, 29, or 79 and a VL CDR3 having an amino acid sequence of SEQ ID NO:6, 16 or 61 to a therapeutically or prophylactically effective serum titer, wherein said effective serum titer is less than 30 µg/ml (and is preferably at least 2 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) after a certain number of days (for example, but not limited to, 20, 25, 30 or 35 days) without any other dosing within that period.

The present invention also provides compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and which have increased *in vivo* half-lives compared to known anti-RSV antibodies as a result of, *e.g.*, one or more modifications in amino acid residues identified to be involved in the interaction between the Fc domain of said antibodies or antibody fragments and the FcRn receptor. In one embodiment, a composition of the invention comprises HL-SYNAGIS or an antigen-binding fragment thereof. In another embodiment, a composition of the invention comprises one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with a higher avidity and/or a



higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen) and which comprise an Fc domain with increased affinity for the FcRn receptor relative to the Fc domain of SYNAGIS®. In accordance with this embodiment, the increased affinity of the Fc domain of said antibodies or antibody fragments results in an *in vivo* half-life of said antibodies or antibody fragments of at least 25 days, preferably at least 30 days, more preferably at least 30 days, and most preferably at least 40 days. In another embodiment, a composition of the invention comprises HL-SYNAGIS or an antigen-binding fragment thereof and one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and which comprise an Fc domain with increased affinity for the FcRn receptor relative to the Fc domain of SYNAGIS®.

The present invention also provides compositions comprising one or more pegylated antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens. In one embodiment, a composition of the invention comprises pegylated SYNAGIS® or a fragment thereof. In another embodiment, a composition of the invention comprises one or more pegylated antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS®. In yet another embodiment, a composition of the invention comprises pegylated SYNAGIS® or an antigen-binding fragment thereof and one or more pegylated antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS®.

The present invention also provides compositions comprising one or more pegylated antibodies or fragments thereof which comprise an Fc domain with increased affinity for the FcRn receptor relative to the Fc domain of SYNAGIS®. In one embodiment, a composition of the invention comprises a pegylated HL-SYNAGIS or an antigen-binding fragment thereof. In another embodiment, a composition of the invention comprises one or more pegylated antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with a higher avidity and/or a higher affinity than known such as, *e.g.*, SYNAGIS® and which comprise an Fc domain with increased affinity for the FcRn receptor relative to the Fc domain of SYNAGIS®.

The present invention encompasses methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human,

comprising administering to said mammal a first dose of a prophylactically or therapeutically effective amount of HL-SYNAGIS or an antigen-binding fragment thereof, wherein said effective amount is approximately 15 mg/kg of said antibodies or fragments thereof which dose results in a serum titer of at least 30  $\mu\text{g/ml}$  at least 30 days after the administration of the first dose and prior to the administration of a subsequent dose. In particular, the present invention provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a human subject, comprising administering to said human subject a first dose of 15 mg/kg of HL-SYNAGIS or an antigen-binding fragment thereof so that said human subject has a serum antibody titer of at least 30  $\mu\text{g/ml}$  at least 30 days after the administration of the first dose of the antibody or antibody fragment and prior to the administration of a subsequent dose.

The present invention also encompasses methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, comprising administering to said mammal a first dose of a prophylactically or therapeutically effective amount of one or more antibodies or fragments thereof which have increased *in vivo* half-lives and which immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen), wherein said effective amount is less than 15 mg/kg (preferably 5 mg/kg or less, more preferably 3 mg/kg or less, and most preferably 1.5 mg/kg or less) of said antibodies or fragments thereof which dose results in a serum titer of less than 30  $\mu\text{g/ml}$  (which is preferably at least 2  $\mu\text{g/ml}$ , more preferably at least 4  $\mu\text{g/ml}$ , and most preferably at least 6  $\mu\text{g/ml}$ ) at least 20 days (preferably at least 25, at least 30, or at least 35 days) after the administration of the first dose and prior to the administration of a subsequent dose. In particular, the present invention provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a human subject, comprising administering to said human subject a first dose of less than 5 mg/kg (preferably 1.5 mg/kg or less) of an antibody or a fragment thereof which has an increased *in vivo* half-life and which immunospecifically binds to a RSV antigen with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV

antigen) so that said human subject has a serum antibody titer of less than 30  $\mu\text{g/ml}$  (which is preferably at least 2  $\mu\text{g/ml}$ , more preferably at least 4  $\mu\text{g/ml}$ , and most preferably at least 6  $\mu\text{g/ml}$ ) at least 25 days (preferably at least 30, at least 35, or at least 40 days) after the administration of the first dose of the antibody or antibody fragment and prior to the  
5 administration of a subsequent dose.

The present invention provides sustained release formulations comprising one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens. In one embodiment, a sustained release formulation comprises SYNAGIS® or a fragment thereof. In another embodiment, a sustained release formulation comprises one or  
10 more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times$   
15  $10^{12} \text{ M}^{-1}$  for a RSV antigen). In another embodiment, a sustained release formulation comprises SYNAGIS® or an antigen-binding fragment thereof and one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ ,  
20 at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen). In another embodiment, HL-SYNAGIS or an antigen-binding fragment thereof is formulated in as sustained release formulation. In yet another embodiment, antibodies or fragments thereof which have higher avidity and/or higher affinity for one or more RSV  
25 antigens than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen) and which comprises an Fc domain with increased affinity for the FcRn receptor relative to the Fc  
30 domain of SYNAGIS® are formulated in sustained release formulations.

The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, comprising administering to said mammal a first dose of a prophylactically or therapeutically effective amount of one or more antibodies or fragments thereof that  
35 immunospecifically bind to one or more RSV antigens in a sustained release formulation,

wherein said effective amount is a dose of 15 mg/kg or less of said antibodies or fragments thereof, which dose, preferably results in a serum titer of at least 2 µg/ml (preferably at least 5 µg/ml, at least 10 µg/ml, at least 20 µg/ml, at least 30 µg/ml, or at least 40 µg/ml) for at least 20 days (preferably at least 25, 30, 35 or 40 days) after the administration of the first  
5 dose and prior to the administration of a subsequent dose.

In one embodiment, a mammal, preferably a human, is administered a first dose of a prophylactically or therapeutically effective amount of SYNAGIS® or an antigen-binding fragment thereof in a sustained release formulation, wherein said effective amount is a dose of approximately 15 mg/kg of SYNAGIS® or an antigen-binding fragment thereof which  
10 dose results in a serum titer of at least 20 µg/ml (preferably at least 30 µg/ml, more preferably at least 40 µg/ml, and most preferably at least 50 µg/ml) for at least 30 days (preferably at least 35 days, more preferably at least 40 days, and most preferably at least 45 days) after the administration of the first dose and prior to the administration of a subsequent dose. In a preferred embodiment, a mammal, preferably a human, is  
15 administered a first dose of a prophylactically or therapeutically effective amount of SYNAGIS® or an antigen-binding fragment thereof in a sustained release formulation, wherein said effective amount is a dose of 15 mg/kg or less of SYNAGIS® or an antigen-binding fragment thereof which dose results in a serum titer of 20 µg/ml (preferably at least 30 µg/ml, more preferably at least 40 µg/ml, and most preferably at least 50 µg/ml) at least  
20 30 days (preferably at least 35 days, more preferably at least 40 days, and most preferably at least 45 days) after the administration of the first dose and prior to the administration of a subsequent dose.

In another embodiment, a mammal, preferably a human, is administered a first dose of a prophylactically or therapeutically effective amount of one or more antibodies or  
25 fragments thereof which immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV  
30 antigen) in a sustained release formulation, wherein said effective amount is a dose of less than 15 mg/kg (preferably 5 mg/kg or less, more preferably 3 mg/kg or less, and most preferably 1.5 mg/kg or less) of said antibodies or antibody fragments which dose results in a serum titer of less than 30 µg/ml (which is preferably at least 2 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) for at least 20 days (preferably at least  
35 25, at least 30, at least 35, or at least 40 days) after the administration of the first dose and

prior to the administration of a subsequent dose. In a preferred embodiment, a mammal, preferably a human, is administered a first dose of a prophylactically or therapeutically effective amount of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen) in a sustained release formulation, wherein said effective amount is a dose of less than 15 mg/kg of said antibodies or antibody fragments which dose results in a serum titer of 10 µg/ml for at least 20 days (preferably at least 25, at least 30, at least 35 or at least 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. In accordance with this embodiment, the prophylactically or therapeutically effective amount of the dose of the antibodies or antibody fragments is approximately 0.5 mg/kg, preferably 1 mg/kg, 1.5 mg/kg, 3 mg/kg, 5 mg/kg, 7.5 mg/kg, 10 mg/kg, 12 mg/kg, or 14 mg/kg. In another preferred embodiment, a mammal, preferably a human, is administered a first dose of a prophylactically or therapeutically effective amount of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with higher avidity and/or higher affinity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen) in a sustained release formulation, wherein said effective amount is a dose of 1.5 mg/kg of said antibodies or antibody fragments which dose results in a serum titer of 10 µg/ml for at least 20 days (preferably at least 25, at least 30, at least 35, or at least 40 days) after the administration of the first dose and prior to the administration of a subsequent dose.

Additionally, the present invention provides sustained release compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens, which sustained release compositions maintain a certain serum titer in a subject for a certain period of time without exceeding a particular serum titer. In one embodiment, a sustained release formulation comprising SYNAGIS® or an antigen-binding fragment thereof maintains a serum titer in a mammal, preferably a human, of approximately 25 µg/ml (preferably 30 µg/ml, more preferably 40 µg/ml, and most preferably 50 µg/ml) without exceeding a serum titer of approximately 100 µg/ml (preferably 75 µg/ml) for at least 20 days (preferably at least 25, 30, 35, or 40 days). In

another embodiment, a sustained release formulation comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with a higher avidity and/or a higher affinity than previously known antibodies such as, *e.g.*, SYNAGIS®, maintains a serum titer in a mammal, preferably a human, of approximately 2  
5  $\mu\text{g/ml}$  (preferably 6  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$ , 20  $\mu\text{g/ml}$ , or 30  $\mu\text{g/ml}$ ) without exceeding a serum titer of approximately 40  $\mu\text{g/ml}$  (preferably 75  $\mu\text{g/ml}$ ) for at least 20 days (preferably at least 25, 30, 35, or 40 days).

The present invention encompasses methods of preventing, treating or ameliorating one or more symptoms of RSV infection in a mammal, preferably a human, by  
10 administering sustained release formulations of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens and which have increased *in vivo* half-lives. In one embodiment, a sustained release formulation comprising HL-SYNAGIS or an antigen-binding fragment thereof is administered to a mammal, preferably a human, to prevent, treat, or ameliorate one or more symptoms associated with a RSV  
15 infection. In another embodiment, a sustained release formulation comprising one or more antibodies or fragments thereof which have higher avidity and/or higher affinity for one or more RSV antigens than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at  
20 least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen) and which comprises an Fc domain with increased affinity for the FcRn receptor relative to the Fc domain of SYNAGIS® are administered to a mammal, preferably a human, to prevent, treat, or ameliorate one or more symptoms associated with a RSV infection.

The present invention also provides pulmonary delivery systems for administering one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens. In particular, the present invention provides compositions for pulmonary delivery, said compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens. SYNAGIS® or an antigen-binding  
30 fragment thereof can be incorporated into compositions for pulmonary delivery. HL-SYNAGIS or an antigen-binding fragment thereof can be incorporated into compositions for pulmonary delivery. One or more antibodies or fragments thereof that bind to one or more RSV antigens with higher affinity and/or higher avidity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  
35  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at

least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen) can be incorporated into compositions for pulmonary delivery. Further, one or more antibodies or fragments thereof which bind to one or more RSV antigens with higher affinity and/or higher avidity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments with an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for a RSV antigen) and which comprise an Fc domain with increased affinity for the FcRn receptor relative to the Fc domain of SYNAGIS® can be incorporated into compositions for pulmonary delivery.

The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection, said methods comprising administering to a mammal, preferably a human, a composition for pulmonary delivery comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens. In particular, the present invention provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection, said methods comprising administering to a mammal, preferably a human, a composition for pulmonary delivery comprising SYNAGIS® or fragments thereof. The present invention also provides methods for preventing, treating or ameliorating one or more symptoms associated with a RSV infection, said methods comprising administering to a mammal, preferably a human, a composition for pulmonary delivery comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with higher affinity and/or higher avidity than known antibodies such as, *e.g.*, SYNAGIS® (*e.g.*, antibodies or antibody fragments having an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for one or more RSV antigens).

In one embodiment, a first dose of a prophylactically or therapeutically effective amount of a composition comprising SYNAGIS® or an antigen-binding fragment thereof is administered to the lungs of a mammal, preferably a human, and results in an antibody concentration of at least 20 ng per mg of lung protein (preferably at least 40 ng/mg, at least 60 ng/mg, at least 80 ng/mg, at least 50 ng/mg, at least 75 ng/mg, at least 100 ng/mg, or at least 150 ng/mg) at least 20 days (preferably at least 25, 30, 35 or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the prophylactically or therapeutically effective amount is a dose of

approximately 0.01 mg/kg, (preferably at least 0.1 mg/kg, at least 1 mg/kg, at least 2 mg/kg, at least 4 mg/kg, at least 5 mg/kg or at least 10 mg/kg) of SYNAGIS® or an antigen-binding fragment thereof.

In another embodiment, a first dose of a prophylactically or therapeutically effective amount of a composition comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with higher affinity and/or higher avidity than known antibodies such as, *e.g.*, SYNAGIS®, (*e.g.*, antibodies or antibody fragments having an affinity of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , or at least  $5 \times 10^{12} \text{ M}^{-1}$  for one or more RSV antigens) is administered to the lungs of a mammal, preferably a human and results in an antibody concentration of 20 ng per mg of lung protein (preferably at least 40 ng/mg, at least 60 ng/mg, at least 80 ng/mg, at least 50 ng/mg, at least 75 ng/mg, at least 100 ng/mg, or at least 150 ng/mg), at least 200 ng/mg, at least 250 ng/mg, at least 500 ng/mg, at least 750 ng/mg, at least 1 µg/mg, at least 2 µg/mg, at least 5 µg/mg, at least 10 µg/mg, at least 15 µg/mg, or at least 25 µg/mg) at least 20 days (preferably at least 25, 30, 35 or 40 days) at least 20 days (preferably at least 25, at least 30, at least 35 or at least 40 days after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the prophylactically effective amount is a dose of approximately 0.001 mg/kg, (preferably at least 0.005 mg/kg, at least 0.01 mg/kg, at least 0.05 mg/kg, at least 0.1 mg/kg, at least 1 mg/kg, at least 2 mg/kg, at least 4 mg/kg, at least 5 mg/kg or at least 10 mg/kg) of said antibodies or antibody fragments.

The present invention further provides detectable or diagnostic compositions comprising using antibodies or fragments thereof that immunospecifically bind to a RSV antigen, and methods for detecting or diagnosing a RSV infection utilizing said compositions.

### 3.1. DEFINITIONS

The term "analog" as used herein refers to a polypeptide that possesses a similar or identical function as a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment but does not necessarily comprise a similar or identical amino acid sequence of a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment, or possess a similar or identical structure of a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment. A polypeptide that has a similar amino acid sequence refers to a polypeptide that satisfies at least one of the following: (a) a



polypeptide having an amino acid sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the amino acid sequence of a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment described herein; (b) a polypeptide encoded by a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence encoding a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment described herein of at least 5 amino acid residues, at least 10 amino acid residues, at least 15 amino acid residues, at least 20 amino acid residues, at least 25 amino acid residues, at least 40 amino acid residues, at least 50 amino acid residues, at least 60 amino acid residues, at least 70 amino acid residues, at least 80 amino acid residues, at least 90 amino acid residues, at least 100 amino acid residues, at least 125 amino acid residues, or at least 150 amino acid residues; and (c) a polypeptide encoded by a nucleotide sequence that is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to the nucleotide sequence encoding a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment described herein. A polypeptide with similar structure to a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment described herein refers to a polypeptide that has a similar secondary, tertiary or quaternary structure of a RSV polypeptide, a fragment of a RSV, an antibody, or antibody fragment described herein. The structure of a polypeptide can be determined by methods known to those skilled in the art, including but not limited to, X-ray crystallography, nuclear magnetic resonance, and crystallographic electron microscopy.

The term "derivative" as used herein refers to a polypeptide that comprises an amino acid sequence of a RSV polypeptide, a fragment of a RSV polypeptide, an antibody that immunospecifically binds to a RSV polypeptide, or an antibody fragment that immunospecifically binds to a RSV polypeptide which has been altered by the introduction of amino acid residue substitutions, deletions or additions. The term "derivative" as used herein also refers to a RSV polypeptide, a fragment of a RSV polypeptide, an antibody that immunospecifically binds to a RSV polypeptide, or an antibody fragment that immunospecifically binds to a RSV polypeptide which has been modified, *i.e.*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups,

proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment may contain one or more non-classical amino acids. A polypeptide derivative possesses a similar or identical function as a RSV polypeptide, a fragment of a RSV polypeptide, an antibody, or antibody fragment described herein.

10 The term "effective neutralizing titer" as used herein refers to the amount of antibody which corresponds to the amount present in the serum of animals (human or cotton rat) that has been shown to be either clinically efficacious (in humans) or to reduce virus by 99% in, for example, cotton rats. The 99% reduction is defined by a specific challenge of, e.g.,  $10^3$  pfu,  $10^4$  pfu,  $10^5$  pfu,  $10^6$  pfu,  $10^7$  pfu,  $10^8$  pfu, or  $10^9$  pfu) of RSV.

15 The term "epitopes" as used herein refers to portions of a RSV polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. An epitope having immunogenic activity is a portion of a RSV polypeptide that elicits an antibody response in an animal. An epitope having antigenic activity is a portion of a RSV polypeptide to which an antibody immunospecifically binds as determined by any method well known in the art, for example, by the immunoassays described herein. Antigenic epitopes need not necessarily be immunogenic.

The term "fragment" as used herein refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of a RSV polypeptide or an antibody that immunospecifically binds to a RSV polypeptide.

30 The term "human infant" as used herein refers to a human less than 24 months, preferably less than 16 months, less than 12 months, less than 6 months, less than 3 months, less than 2 months, or less than 1 month of age.

The term "human infant born prematurely" as used herein refers to a human born at less than 40 weeks gestational age, preferably less than 35 weeks gestational age, who is less than 6 months old, preferably less than 3 months old, more preferably less than 2 months old, and most preferably less than 1 month old.

5 An "isolated" or "purified" antibody or fragment thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes  
10 preparations of an antibody or antibody fragment in which the antibody or antibody fragment is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, an antibody or antibody fragment that is substantially free of cellular material includes preparations of antibody or antibody fragment having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the antibody or antibody fragment is  
15 recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the antibody or antibody fragment is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the  
20 protein. Accordingly such preparations of the antibody or antibody fragment have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the antibody or antibody fragment of interest. In a preferred embodiment, antibodies of the invention or fragments thereof are isolated or purified.

An "isolated" nucleic acid molecule is one which is separated from other nucleic  
25 acid molecules which are present in the natural source of the nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. In a preferred embodiment, nucleic acid molecules encoding  
30 antibodies of the invention or fragments thereof are isolated or purified.

The term "fusion protein" as used herein refers to a polypeptide that comprises an amino acid sequence of an antibody or fragment thereof and an amino acid sequence of a heterologous polypeptide (*e.g.*, a non-anti-RSV antigen antibody).

The term "high potency" as used herein refers to antibodies or fragments thereof that  
35 exhibit high potency as determined in various assays for biological activity (*e.g.*,

neutralization of RSV) such as those described herein. For example, high potency antibodies of the present invention or fragments thereof have an  $EC_{50}$  value less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM, less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM as measured by a microneutralization assay described herein. Further, high potency antibodies of the present invention or fragments thereof result in at least a 75%, preferably at least a 95% and more preferably a 99% lower RSV titer in a cotton rat 5 days after challenge with  $10^5$  pfu relative to a cotton rat not administered said antibodies or antibody fragments. In certain embodiments of the invention, high potency antibodies of the present invention or fragments thereof exhibit a high affinity and/or high avidity for one or more RSV antigens (*e.g.*, antibodies or antibody fragments having an affinity of at least  $2 \times 10^8$   $M^{-1}$ , at least  $2.5 \times 10^8$   $M^{-1}$ , at least  $5 \times 10^8$   $M^{-1}$ , at least  $10^9$   $M^{-1}$ , at least  $5 \times 10^9$   $M^{-1}$ , at least  $10^{10}$   $M^{-1}$ , at least  $5 \times 10^{10}$   $M^{-1}$ , at least  $10^{11}$   $M^{-1}$ , at least  $5 \times 10^{11}$   $M^{-1}$ , at least  $10^{12}$   $M^{-1}$ , or at least  $5 \times 10^{12}$   $M^{-1}$  for one or more RSV antigens).

The term "host" as used herein refers to a mammal, preferably a human.

The term "host cell" as used herein refers to the particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

In certain embodiments of the invention, a "prophylactically effective serum titer" is the serum titer in a mammal, preferably a human, that reduces the incidence of a RSV infection in said mammal. Preferably, the prophylactically effective serum titer reduces the incidence of RSV infections in humans with the greatest probability of complications resulting from RSV infection (*e.g.*, a human with cystic fibrosis, bronchopulmonary dysplasia, congenital heart disease, congenital immunodeficiency or acquired immunodeficiency, a human who has had a bone marrow transplant, a human infant, or an elderly human). In certain other embodiments of the invention, a "prophylactically effective serum titer" is the serum titer in a cotton rat that results in a RSV titer 5 days after challenge with  $10^5$  pfu that is 99% lower than the RSV titer 5 days after challenge with  $10^5$  pfu of RSV in a cotton rat not administered an antibody or antibody fragment that immunospecifically binds to a RSV antigen.

In certain embodiments of the invention, a "therapeutically effective serum titer" is the serum titer in a mammal, preferably a human, that reduces the severity, the duration and/or the symptoms associated with a RSV infection in said mammal. Preferably, the

therapeutically effective serum titer reduces the severity, the duration and/or the number symptoms associated with RSV infections in humans with the greatest probability of complications resulting from a RSV infection (*e.g.*, a human with cystic fibrosis, bronchopulmonary dysplasia, congenital heart disease, congenital immunodeficiency or acquired immunodeficiency, a human who has had a bone marrow transplant, a human infant, or an elderly human). In certain other embodiments of the invention, a “therapeutically effective serum titer” is the serum titer in a cotton rat that results in a RSV titer 5 days after challenge with  $10^5$  pfu that is 99% lower than the RSV titer 5 days after challenge with  $10^5$  pfu of RSV in a cotton rat not administered an antibody or antibody fragment that immunospecifically binds to a RSV antigen.

As used herein, “HL-SYNAGIS” is SYNAGIS® with one or more modifications in amino acid residues identified to be involved in the interaction between the Fc domain of SYNAGIS® and the FcRn receptor which results in an increase in the *in vivo* half-life of SYNAGIS® to greater than 21 days. An antigen-binding fragment of HL-SYNAGIS is a fragment of SYNAGIS® which immunospecifically binds to RSV F glycoprotein and has one or more modifications in amino acid residues identified to be involved in the interaction between the Fc domain of SYNAGIS® and the FcRn receptor, wherein said modifications result in an increase in the *in vivo* half-life of the antigen-binding fragment. In accordance with the invention, HL-SYNAGIS or an antigen-binding fragment thereof has an *in vivo* half-life of at least 25 days, preferably at least 30 days, more preferably at least 35 days, and most preferably at least 40 days.

The term “RSV antigen” refers to a RSV polypeptide or fragment thereof to which an antibody or antibody fragment immunospecifically binds. An RSV antigen also refers to an analog or derivative of a RSV polypeptide or fragment thereof to which an antibody or antibody fragment immunospecifically binds.

The term “serum titer” as used herein refers to an average serum titer in a population of least 10, preferably at least 20, and most preferably at least 40 subjects.

The term “antibodies or fragments that immunospecifically bind to a RSV antigen” as used herein refers to antibodies or fragments thereof that specifically bind to a RSV polypeptide or a fragment of a RSV polypeptide and do not non-specifically bind to other polypeptides. Antibodies or fragments that immunospecifically bind to a RSV polypeptide or fragment thereof may have cross-reactivity with other antigens. Preferably, antibodies or fragments that immunospecifically bind to a RSV polypeptide or fragment thereof do not cross-react with other antigens. Antibodies or fragments that immunospecifically bind to a

RSV polypeptide can be identified, for example, by immunoassays or other techniques known to those of skill in the art.

To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, wordlength=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present invention. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score=50, wordlength=3 to obtain amino acid sequences homologous to a protein molecule of the present invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, <http://www.ncbi.nlm.nih.gov>). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid

sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent  
5 identity, typically only exact matches are counted.

#### 4. DESCRIPTION OF THE FIGURES

Figures 1A-1B show the amino acid sequences of the (A) light chain variable region and (B) heavy chain variable region of a high affinity monoclonal antibody that binds to a  
10 RSV antigen the potency of which can be increased by methods described herein or in Applicants' copending applications Serial Nos. 60/168,426 and 60/186,252. For reference purposes, this is the amino acid sequence of the SYNAGIS® antibody disclosed in Johnson et al, 1997, J. Infect. Dis. 176:1215-1224 and U.S. Patent No. 5,824,307. Here, the CDR regions are underlined while non-underlined residues form the framework regions of the  
15 variable regions of each antibody. In this antibody, the CDRs are derived from a mouse antibody while the framework regions are derived from a human antibody. The constant regions (not shown) are also derived from a human antibody.

Figures 2A-2B show the (A) light chain variable region and (B) heavy light chain variable region for an antibody sequence. CDR regions are underlined. This sequence  
20 differs from the sequence disclosed in Figures 1A-1B in the first 4 residues of VH CDR1 of the light chain, residue 103 of the light chain and residue 112 of the heavy chain.

#### 5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods of preventing, neutralizing, treating and  
25 ameliorating one or more symptoms associated with a RSV infection in a subject comprising administering to said subject one or more antibodies which immunospecifically bind to one or more RSV antigens with high affinity and/or high avidity and/or have a longer serum half-life. The high affinity and/or high avidity of the antibodies of the invention enable the use of lower doses of said antibodies than previously thought to be  
30 effective for the prevention, neutralization, treatment and the amelioration of symptoms associated with RSV infection. The use of lower doses of antibodies which immunospecifically bind to one or more RSV antigens reduces the likelihood of adverse effects, as well as providing a more effective prophylaxis. Further, the high affinity and/or high avidity of the antibodies of the invention enable less frequent administration of said  
35

antibodies than previously thought to be necessary for the prevention, neutralization, treatment and the amelioration of symptoms associated with RSV infection.

The present invention also provides methods of preventing, neutralizing, treating and ameliorating one or more symptoms associated with a RSV infection in a subject comprising administering to said subject one or more antibodies which immunospecifically bind to one or more RSV antigens, said antibodies having a longer half-life than other previously known antibodies.

The present invention also provides improved methods of administering one or more antibodies which immunospecifically bind to one or more RSV antigens to a subject, said methods enable lower doses of said antibodies to be administered to the subject while achieving serum titers effective for the prevention, neutralization, treatment and amelioration of one or more symptoms associated with RSV infection. The present invention encompasses methods of delivering one or more antibodies which immunospecifically bind to one or more RSV antigens directly to the site of RSV infection. In particular, the invention encompasses pulmonary delivery of one or more antibodies which immunospecifically bind to one or more RSV antigens. The improved methods of delivering of one or more antibodies which immunospecifically bind to one or more RSV antigens reduces the dosage and frequency of administration of said antibodies to a subject.

The present invention is based, in part, upon achieving or inducing a serum titer of 1  $\mu\text{g/ml}$  or less, preferably 2  $\mu\text{g/ml}$  or less, 5  $\mu\text{g/ml}$  or less, 6  $\mu\text{g/ml}$  or less, 10  $\mu\text{g/ml}$  or less, 15  $\mu\text{g/ml}$  or less, 20  $\mu\text{g/ml}$  or less, or 25  $\mu\text{g/ml}$  or less of an antibody or fragment thereof that immunospecifically binds to a respiratory syncytial virus (RSV) antigen in a mammal with higher affinity and/or higher avidity than previously known antibodies, while reducing or avoiding adverse affects. Preferably a serum titer or serum titer of 1  $\mu\text{g/ml}$  or less, preferably 2  $\mu\text{g/ml}$  or less, 5  $\mu\text{g/ml}$  or less, 6  $\mu\text{g/ml}$  or less, 10  $\mu\text{g/ml}$  or less, 15  $\mu\text{g/ml}$  or less, 20  $\mu\text{g/ml}$  or less, or 25  $\mu\text{g/ml}$  or less is achieved approximately 20 days (preferably 25, 30, 35 or 40 days) after administration of a first dose of antibodies or fragments thereof which immunospecifically bind to a RSV antigen and without administration of any other doses of said antibodies or fragments thereof.

The present invention provides methods of achieving or inducing a serum titer of at least 30  $\mu\text{g/ml}$ , at least 40  $\mu\text{g/ml}$ , at least 50  $\mu\text{g/ml}$ , at least 75  $\mu\text{g/ml}$ , at least 100  $\mu\text{g/ml}$ , at least 125  $\mu\text{g/ml}$ , at least 150  $\mu\text{g/ml}$ , at least 175  $\mu\text{g/ml}$ , at least 200  $\mu\text{g/ml}$ , at least 225  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , at least 275  $\mu\text{g/ml}$ , at least 300  $\mu\text{g/ml}$ , at least 325  $\mu\text{g/ml}$ , at least 350  $\mu\text{g/ml}$ , at least 375  $\mu\text{g/ml}$ , or at least 400  $\mu\text{g/ml}$  of an antibody or fragment thereof that immunospecifically binds to a respiratory syncytial virus (RSV) antigen in a mammal, while



reducing or avoiding adverse affects. Preferably a serum titer or serum titer of at least 30  $\mu\text{g/ml}$ , preferably at least 40  $\mu\text{g/ml}$ , at least 50  $\mu\text{g/ml}$ , at least 75  $\mu\text{g/ml}$ , at least 100  $\mu\text{g/ml}$ , at least 125  $\mu\text{g/ml}$ , at least 150  $\mu\text{g/ml}$ , at least 175  $\mu\text{g/ml}$ , at least 200  $\mu\text{g/ml}$ , at least 225  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , at least 275  $\mu\text{g/ml}$ , at least 300  $\mu\text{g/ml}$ , at least 325  $\mu\text{g/ml}$ , at least 350  $\mu\text{g/ml}$ , at least 375  $\mu\text{g/ml}$ , or at least 400  $\mu\text{g/ml}$  is achieved approximately 30 days after administration of a first dose of antibodies or fragments thereof which immunospecifically bind to a RSV antigen and without administration of any other doses of said antibodies or fragments thereof.

In a specific embodiment, a serum titer in a non-primate mammal of at least 40  $\mu\text{g/ml}$ , preferably at least 80  $\mu\text{g/ml}$ , at least 100  $\mu\text{g/ml}$ , at least 120  $\mu\text{g/ml}$ , at least 150  $\mu\text{g/ml}$ , at least 200  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , or at least 300  $\mu\text{g/ml}$ , of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens is achieved at least 1 day after administering a dose of less than 2.5 mg/kg, preferably less than 1 mg/kg, or less than 0.5 mg/kg of the antibodies or antibody fragments to the non-primate mammal. In another embodiment, a serum titer in a non-primate mammal of at least 150  $\mu\text{g/ml}$ , preferably at least 200  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , at least 300  $\mu\text{g/ml}$ , at least 350  $\mu\text{g/ml}$ , or at least 400  $\mu\text{g/ml}$  of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens is achieved at least 1 day after administering a dose of approximately 5 mg/kg of the antibodies or antibody fragments to the non-primate mammal.

In another embodiment, a serum titer in a primate of at least 40  $\mu\text{g/ml}$ , preferably at least 80  $\mu\text{g/ml}$ , at least 100  $\mu\text{g/ml}$ , at least 120  $\mu\text{g/ml}$ , at least 150  $\mu\text{g/ml}$ , at least 200  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , or at least 300  $\mu\text{g/ml}$  of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens is achieved at least 30 days after administering a first dose of less than 5 mg/kg, preferably less than 3 mg/kg, less than 1 mg/kg, or less than 0.5 mg/kg of the antibodies or fragments thereof to the primate. In yet another embodiment, a serum titer in a primate of at least 200  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , at least 300  $\mu\text{g/ml}$ , at least 350  $\mu\text{g/ml}$ , or at least 400  $\mu\text{g/ml}$  of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens is achieved at least 30 days after administering a first dose of approximately 15 mg/kg of the antibodies or fragments thereof to the primate. In accordance with these embodiments, the primate is preferably a human.

The present invention provides methods for preventing, treating, or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, said methods comprising administering a first dose to said mammal of a prophylactically or

therapeutically effective amount of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, wherein said effective amount is less than 15 mg/kg of said antibodies or fragments thereof and which results in a serum titer of greater than 40  $\mu\text{g/ml}$  30 days after the first administration and prior to any subsequent administration. In one embodiment, RSV infection in a human subject is prevented or treated, or one or more symptoms associated with RSV infection is ameliorated by administering a first dose of less than 10 mg/kg, preferably less than 5 mg/kg, less than 3 mg/kg, or less than 1 mg/kg of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens so that a serum antibody titer of at least 40  $\mu\text{g/ml}$ , preferably at least 80  $\mu\text{g/ml}$ , or at least 120  $\mu\text{g/ml}$ , at least 150  $\mu\text{g/ml}$ , at least 200  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , or at least 300  $\mu\text{g/ml}$  is achieved 30 days after the administration of the first dose of the antibodies or antibody fragments and prior to the administration of a subsequent dose. In another embodiment, RSV infection in a human subject is prevented or treated, or one or more symptoms associated with a RSV infection is ameliorated by administering a first dose of approximately 15 mg/kg of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens so that a serum antibody titer of at least 75  $\mu\text{g/ml}$ , preferably at least 100  $\mu\text{g/ml}$ , at least 200  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , at least 300  $\mu\text{g/ml}$ , at least 350  $\mu\text{g/ml}$ , or at least 400  $\mu\text{g/ml}$  is achieved 30 days after the administration of the first dose of the antibodies or antibody fragments and prior to the administration of a subsequent dose. In yet another embodiment, RSV infection in a human subject is prevented or treated, or one or more symptoms associated with a RSV infection is ameliorated by administering a first dose of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens such that a prophylactically or therapeutically effective serum titer of less than 10  $\mu\text{g/ml}$ , preferably less than 5  $\mu\text{g/ml}$ , less than 3  $\mu\text{g/ml}$ , less than 1  $\mu\text{g/ml}$ , or less than 0.5  $\mu\text{g/ml}$  is achieved no more than 30 days after administering the antibodies or antibody fragments. In accordance with this embodiment, the first dose of one or more antibodies or fragments thereof is less than 10 mg/kg, preferably less than 5 mg/kg, less than 1 mg/kg, or less than 0.5 mg/kg.

The present invention provides antibodies or fragments thereof which immunospecifically bind to a RSV antigen with an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$ . Preferably, the antibodies or antibody fragments have

a higher affinity for a RSV antigen than SYNAGIS® does for the RSV F glycoprotein. The present invention also provides pharmaceutical compositions comprising one or more antibodies which immunospecifically bind to a RSV antigen with an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9$   
5  $\text{M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$ .

The present invention also provides antibodies or fragments thereof which immunospecifically bind to a RSV antigen with a higher avidity than any previously known  
10 antibodies or fragments thereof. Preferably, the antibodies or antibody fragments have higher avidity for a RSV antigen than SYNAGIS® has for the RSV F glycoprotein. The present invention also provides antibodies or fragments thereof that immunospecifically bind to a RSV antigen which have a higher affinity for a RSV antigen than any previously known antibodies or fragments thereof. The present invention also provides pharmaceutical  
15 compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to a RSV antigen with a higher avidity than any previously known antibodies or fragments thereof.

The present invention also provides for antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at  
20 least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$  and which have a higher avidity for one or more RSV antigens than any previously known antibodies or fragments thereof such as, *e.g.*,  
25 SYNAGIS®. The present invention further provides pharmaceutical compositions comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8$   
 $\text{M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times$   
 $10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at  
30 least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$  and which have a higher avidity for one or more RSV antigens than any previously known antibodies or fragments thereof such as, *e.g.*, SYNAGIS®.

The present invention provides methods of achieving a certain serum titer (preferably a serum titer  $1 \mu\text{g/ml}$  or less,  $2 \mu\text{g/ml}$  or less,  $5 \mu\text{g/ml}$  or less,  $6 \mu\text{g/ml}$  or less,  $10$   
35  $\mu\text{g/ml}$  or less,  $15 \mu\text{g/ml}$  or less,  $20 \mu\text{g/ml}$  or less, or  $25 \mu\text{g/ml}$  or less) of antibodies or

fragments thereof that immunospecifically bind to one or more RSV antigens in a mammal, said methods comprising administering to said mammal one or more antibodies or fragments thereof that have an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$  for said RSV antigens. Preferably, the antibodies or antibody fragments have a higher affinity for a RSV antigen than SYNAGIS® does for the RSV F glycoprotein.

The present invention also provides methods of achieving a certain serum titer of antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens in a mammal, said methods comprising administering to said mammal one or more antibodies or fragments thereof that have a higher avidity for said RSV antigens than any previously known antibodies or antibody fragments. Preferably, the antibodies or antibody fragments have higher avidity for a RSV antigen than SYNAGIS® has for the RSV F glycoprotein.

The present invention also provides methods of achieving a certain serum titer of antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens in mammal, said methods comprising administering to said mammal one or more antibodies or fragments thereof that have an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$  for one or more RSV antigens and have a higher avidity than any previously known antibodies or antibody fragments for said RSV antigens.

The present invention also provides methods of neutralizing RSV using antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens and which have an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$  for said RSV antigens. Preferably, the antibodies or antibody fragments have a higher affinity for a RSV antigen than SYNAGIS® does for the RSV F glycoprotein. The present invention also provides methods of neutralizing RSV using antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens and which have a higher avidity for said RSV antigens than any previously known antibodies or antibody fragments. Preferably,

the antibodies or antibody fragments have a higher avidity for a RSV antigen than SYNAGIS® does for the RSV F glycoprotein. The present invention also provides methods of neutralizing RSV using antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8$   
 5  $\text{M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$  and which have a higher avidity for said RSV antigens than any previously known antibodies or antibody fragments. The higher affinity and/or higher  
 10 avidity that these antibodies or antibody fragments have for a RSV antigen results in a lower concentration of these antibodies or antibody fragments necessary to achieve neutralization of RSV than previously known.

The present invention also provides methods for preventing, treating or ameliorating one or more symptoms of RSV infection in a mammal, said methods comprising  
 15 administering to said mammal one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens and which have an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at  
 20 least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$  for said RSV antigens.

Preferably, the antibodies or antibody fragments have a higher affinity for a RSV antigen than SYNAGIS® does for the RSV F glycoprotein. The present invention also provides of methods preventing, treating or ameliorating one or more symptoms of RSV infection in a mammal, said methods comprising administering to said mammal one or more antibodies or  
 25 fragments thereof that immunospecifically bind to one or more RSV antigen and which have a higher avidity for said RSV antigen than any previously known antibodies or antibody fragments. Preferably, the antibodies or antibody fragments have a higher avidity for a RSV antigen than SYNAGIS® does for the RSV F glycoprotein. The present invention further provides methods of preventing, treating or ameliorating one or more  
 30 symptoms of RSV infection in a mammal, said methods comprising administering to said mammal one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with an affinity constant of at least  $2 \times 10^8 \text{ M}^{-1}$ , at least  $2.5 \times 10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  
 35  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at

least  $5 \times 10^{15} \text{ M}^{-1}$  for said RSV antigen and which have a higher avidity for said RSV antigens than any previously known antibodies or antibody fragments. The higher affinity and/or higher avidity that these antibodies or antibody fragments have for a RSV antigen results in lower and/or less frequent doses of these antibodies or antibody fragments to achieve a prophylactic or therapeutic effect in a mammal, preferably a human, than previously known.

The present invention provides methods for preventing, treating, or ameliorating one or more symptoms associated with a RSV infection in a mammal, preferably a human, said methods comprising administering a first dose to said mammal of a prophylactically or therapeutically effective amount of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with a higher avidity and/or affinity than previously known antibodies such as SYNAGIS®, wherein said effective amount is less than 15 mg/kg of said antibodies or fragments thereof and which results in a serum titer less than 30 µg/ml (which is preferably at least 2 µg/ml, more preferably at least 4 µg/ml, and most preferably at least 6 µg/ml) 30 days after the first administration and prior to any subsequent administration. In one embodiment, RSV infection in a human subject is prevented or treated, or one or more symptoms in a human subject is ameliorated by administering a first dose of less than 10 mg/kg, preferably less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg, or less than 0.5 mg/kg of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with a higher avidity and/or affinity than previously known antibodies such as SYNAGIS® so that a serum antibody titer of at least 6 µg/ml, preferably at least 10 µg/ml, at least 25 µg/ml, at least 30 µg/ml, at least 40 µg/ml at least 80 µg/ml, or at least 120 µg/ml, at least 150 µg/ml, at least 200 µg/ml, at least 250 µg/ml, or at least 300 µg/ml is achieved 30 days after the administration of the first dose of the antibodies or antibody fragments and prior to the administration of a subsequent dose.

The present invention provides antibodies or fragments thereof comprising a variable heavy ("VH") domain having an amino acid sequence of any VH domain listed in Table 2, and pharmaceutical compositions comprising said antibodies or antibody fragments. The present invention also provides antibodies or fragments thereof comprising one or more VH CDRs having the amino acid sequence of one or more VH CDRs listed in Table 2 and/or Table 3, and pharmaceutical compositions comprising said antibodies or antibody fragments. The present invention also provides antibodies or fragments thereof comprising a variable light ("VL") domain having the amino acid sequence of any VL domain listed in Table 2, and pharmaceutical compositions comprising said antibodies or

antibody fragments. The present invention also provides antibodies or fragments thereof comprising one or more VL CDRs having the amino acid sequence of one or more VL CDRs listed in Table 2 and/or Table 3, and pharmaceutical compositions comprising said antibodies or antibody fragments. The present invention also provides antibodies or  
5 fragments thereof comprising a VH domain having the amino acid sequence any VH domain listed in Table 2 and a VL domain having the amino acid sequence of any VL domain listed in Table 2, and pharmaceutical compositions comprising said antibodies or antibody fragments. The present invention provides antibodies or fragments thereof comprising one or more VH CDRs having the amino acid sequence one or more VH CDRs  
10 listed in Table 2 and/or Table 3 and one or more VL CDRs having the amino acid sequence of one or more VL CDRs listed in Table 2 and/or Table 3. The present invention encompasses pharmaceutical compositions comprising said antibodies or antibody fragments. Preferably, said antibodies or antibody fragments immunospecifically bind to one or more RSV antigens.

15 The present invention encompasses methods for preventing, treating, neutralizing and ameliorating one or more symptoms using one or more antibodies comprising a variable heavy ("VH") domain having an amino acid sequence of any VH domain listed in Table 2. The present invention also encompasses methods for preventing, treating, neutralizing and ameliorating one or more symptoms using one or more antibodies comprising one or more  
20 VH CDRs having the amino acid sequence of one or more VH CDRs listed in Table 2 and/or Table 3. The present invention also encompasses methods for preventing, treating, neutralizing and ameliorating one or more symptoms using one or more antibodies comprising a variable light ("VL") domain having the amino acid sequence of any VL domain listed in Table 2. The present invention also encompasses methods for preventing,  
25 treating, neutralizing and ameliorating one or more symptoms using one or more antibodies comprising one or more VL CDRs having the amino acid sequence of one or more VL CDRs listed in Table 2 and/or Table 3. The present invention also encompasses methods for preventing, treating, neutralizing and ameliorating one or more symptoms using one or more antibodies comprising a VH domain having the amino acid sequence any VH domain  
30 listed in Table 2 and a VL domain having the amino acid sequence of any VL domain listed in Table 2. The present invention further encompasses methods for preventing, treating, neutralizing and ameliorating one or more symptoms using one or more antibodies comprising one or more VH CDRs having the amino acid sequence one or more VH CDRs listed in Table 2 and/or Table 3 and one or more VL CDRs having the amino acid sequence  
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of one or more VL CDRs listed in Table 2 and/or Table 3. Preferably, said antibodies or antibody fragments immunospecifically bind to one or more RSV antigens.

The present invention encompasses antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with increased *in vivo* half-lives. In particular, the present invention encompasses HL-SYNAGIS and antigen-binding fragments thereof. The present invention also encompasses novel antibodies or fragments thereof described herein which immunospecifically bind to one or more RSV antigens and have an Fc domain with a higher affinity for the FcRn receptor than the Fc domain of SYNAGIS®.

The present invention also encompasses methods for the prevention, neutralization, treatment or amelioration of one or more symptoms associated with a RSV infection using antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with increased *in vivo* half-lives. In particular, the invention encompasses methods for the prevention, neutralization, treatment or amelioration of one or more symptoms associated with a RSV infection using HL-SYNAGIS or an antigen-binding fragment thereof. The invention also encompasses methods for the prevention, neutralization, treatment or amelioration of one or more symptoms associated with a RSV infection using novel antibodies or fragments thereof described herein which immunospecifically bind to one or more RSV antigens and have an Fc domain with a higher affinity for the FcRn receptor than the Fc domain of SYNAGIS®.

The present invention provides sustained release formulations of antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens for the prevention, neutralization, treatment or amelioration of one or more symptoms associated with a RSV infection. In particular, the present invention provides sustained release formulations of SYNAGIS® or fragments thereof for the prevention, neutralization, treatment or amelioration of one or more symptoms associated with a RSV infection. The present invention also provides sustained release formulations of one or more novel antibodies or fragments thereof described herein which immunospecifically bind to one or more RSV antigens for the prevention, neutralization, treatment or amelioration of one or more symptoms associated with a RSV infection.

The present invention also provides methods of administering compositions comprising antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens to the site of a RSV infection in a subject. In particular, the present invention provides compositions comprising one or more antibodies or fragments thereof for pulmonary delivery to a subject.

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The present invention provides compositions comprising one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, and methods for detecting or diagnosing a RSV infection utilizing said antibodies or antibody fragments.

5                   **5.1.    Antibodies**

It should be recognized that antibodies that immunospecifically bind to a RSV antigen are known in the art. For example, SYNAGIS® is a humanized monoclonal antibody presently used for the prevention of RSV infection in pediatric patients. The present invention encompasses novel formulations for administration of SYNAGIS® and  
10 other known anti-RSV antibodies and novel doses of SYNAGIS® and other known anti-RSV antibodies, as discussed herein.

In addition, the invention encompasses novel antibodies, fragments and other biological or macromolecules which immunospecifically bind to one or more RSV antigens. With respect to these novel agents, the invention further encompasses novel modes of  
15 administration, doses, dosing and uses based, in part, upon their unique therapeutic profiles and potency.

Set forth below, is a more detailed description of the antibodies encompassed within the various aspects of the invention.

The present invention provides antibodies or fragments thereof that  
20 immunospecifically bind to one or more RSV antigens. The present invention provides antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens. Preferably, the antibodies of the invention or fragments thereof immunospecifically bind to one or more RSV antigens regardless of the strain of RSV. The present invention also provides antibodies or fragments thereof that differentially or preferentially bind to RSV  
25 antigens from one strain of RSV versus another RSV strain. In a specific embodiment, the antibodies of the invention or fragments thereof immunospecifically bind to the RSV F glycoprotein, G glycoprotein or SH protein. In a preferred embodiment, the antibodies present invention or fragments thereof immunospecifically bind to the RSV F glycoprotein. In another preferred embodiment, the antibodies of the present invention or fragments  
30 thereof bind to the A, B, or C antigenic sites of the RSV F glycoprotein.

Antibodies of the invention include, but are not limited to, monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies  
35 to antibodies of the invention), and epitope-binding fragments of any of the above. In

particular, antibodies of the present invention include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds to a RSV antigen. The immunoglobulin molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, IgG<sub>4</sub>, IgA<sub>1</sub> and IgA<sub>2</sub>) or subclass of immunoglobulin molecule.

The antibodies of the invention may be from any animal origin including birds and mammals (*e.g.*, human, murine, donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken). Preferably, the antibodies of the invention are human or humanized monoclonal antibodies. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from mice that express antibodies from human genes.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a RSV polypeptide or may be specific for both a RSV polypeptide as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, *e.g.*, PCT publications WO 93/17715, WO 92/08802, WO 91/00360, and WO 92/05793; Tutt, et al., J. Immunol. 147:60-69(1991); U.S. Patent Nos. 4,474,893, 4,714,681, 4,925,648, 5,573,920, and 5,601,819; and Kostelny et al., J. Immunol. 148:1547-1553 (1992).

The present invention provides for antibodies or fragments thereof that exhibit a high potency in an assay described herein. High potency antibodies or fragments thereof can be produced by methods disclosed in copending U.S. patent application Serial Nos. 60/168,426 and 60/186,252 and methods described herein. For example, high potency antibodies can be produced by genetically engineering appropriate antibody gene sequences and expressing the antibody sequences in a suitable host. The antibodies produced can be screened to identify antibodies with, *e.g.*, high  $k_{on}$  values in a BIAcore assay.

The present invention provides for antibodies or fragments thereof that have a high binding affinity for one or more RSV antigens. In a specific embodiment, an antibody of the present invention or fragment thereof has an association rate constant or  $k_{on}$  rate (antibody (Ab) + antigen (Ag)  $\xrightarrow{k_{on}}$  Ab-Ag) of at least  $10^5 \text{ M}^{-1}\text{s}^{-1}$ , at least  $5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ , at least  $10^6 \text{ M}^{-1}\text{s}^{-1}$ , at least  $5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ , at least  $10^7 \text{ M}^{-1}\text{s}^{-1}$ , at least  $5 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ , or at least  $10^8 \text{ M}^{-1}\text{s}^{-1}$ . In a preferred embodiment, an antibody of the present invention or fragment thereof has a  $k_{on}$  of at least  $2 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ , at least  $5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ , at least  $10^6 \text{ M}^{-1}\text{s}^{-1}$ , at least  $5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$ , at least  $10^7 \text{ M}^{-1}\text{s}^{-1}$ , at least  $5 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$ , or at least  $10^8 \text{ M}^{-1}\text{s}^{-1}$ .

In another embodiment, an antibody of the present invention or fragment thereof has a  $k_{\text{off}}$  rate (antibody (Ab) + antigen (Ag)  $\xrightleftharpoons{K_{\text{off}}} \text{Ab-Ag}$ ) of less than  $10^{-1} \text{ s}^{-1}$ , less than  $5 \times 10^{-1} \text{ s}^{-1}$ , less than  $10^{-2} \text{ s}^{-1}$ , less than  $5 \times 10^{-2} \text{ s}^{-1}$ , less than  $10^{-3} \text{ s}^{-1}$ , less than  $5 \times 10^{-3} \text{ s}^{-1}$ , less than  $10^{-4} \text{ s}^{-1}$ , less than  $5 \times 10^{-4} \text{ s}^{-1}$ , less than  $10^{-5} \text{ s}^{-1}$ , less than  $5 \times 10^{-5} \text{ s}^{-1}$ , less than  $10^{-6} \text{ s}^{-1}$ , less than  $5 \times 10^{-6} \text{ s}^{-1}$ , less than  $10^{-7} \text{ s}^{-1}$ , less than  $5 \times 10^{-7} \text{ s}^{-1}$ , less than  $10^{-8} \text{ s}^{-1}$ , less than  $5 \times 10^{-8} \text{ s}^{-1}$ , less than  $10^{-9} \text{ s}^{-1}$ , less than  $5 \times 10^{-9} \text{ s}^{-1}$ , or less than  $10^{-10} \text{ s}^{-1}$ . In a preferred embodiment, an antibody of the present invention or fragment thereof has a  $k_{\text{on}}$  of less than  $5 \times 10^{-4} \text{ s}^{-1}$ , less than  $10^{-5} \text{ s}^{-1}$ , less than  $5 \times 10^{-5} \text{ s}^{-1}$ , less than  $10^{-6} \text{ s}^{-1}$ , less than  $5 \times 10^{-6} \text{ s}^{-1}$ , less than  $10^{-7} \text{ s}^{-1}$ , less than  $5 \times 10^{-7} \text{ s}^{-1}$ , less than  $10^{-8} \text{ s}^{-1}$ , less than  $5 \times 10^{-8} \text{ s}^{-1}$ , less than  $10^{-9} \text{ s}^{-1}$ , less than  $5 \times 10^{-9} \text{ s}^{-1}$ , or less than  $10^{-10} \text{ s}^{-1}$ .

In another embodiment, an antibody of the present invention or fragment thereof has an affinity constant or  $K_a$  ( $k_{\text{on}}/k_{\text{off}}$ ) of at least  $10^2 \text{ M}^{-1}$ , at least  $5 \times 10^2 \text{ M}^{-1}$ , at least  $10^3 \text{ M}^{-1}$ , at least  $5 \times 10^3 \text{ M}^{-1}$ , at least  $10^4 \text{ M}^{-1}$ , at least  $5 \times 10^4 \text{ M}^{-1}$ , at least  $10^5 \text{ M}^{-1}$ , at least  $5 \times 10^5 \text{ M}^{-1}$ , at least  $10^6 \text{ M}^{-1}$ , at least  $5 \times 10^6 \text{ M}^{-1}$ , at least  $10^7 \text{ M}^{-1}$ , at least  $5 \times 10^7 \text{ M}^{-1}$ , at least  $10^8 \text{ M}^{-1}$ , at least  $5 \times 10^8 \text{ M}^{-1}$ , at least  $10^9 \text{ M}^{-1}$ , at least  $5 \times 10^9 \text{ M}^{-1}$ , at least  $10^{10} \text{ M}^{-1}$ , at least  $5 \times 10^{10} \text{ M}^{-1}$ , at least  $10^{11} \text{ M}^{-1}$ , at least  $5 \times 10^{11} \text{ M}^{-1}$ , at least  $10^{12} \text{ M}^{-1}$ , at least  $5 \times 10^{12} \text{ M}^{-1}$ , at least  $10^{13} \text{ M}^{-1}$ , at least  $5 \times 10^{13} \text{ M}^{-1}$ , at least  $10^{14} \text{ M}^{-1}$ , at least  $5 \times 10^{14} \text{ M}^{-1}$ , at least  $10^{15} \text{ M}^{-1}$ , or at least  $5 \times 10^{15} \text{ M}^{-1}$ . In yet another embodiment, an antibody or fragment thereof has a dissociation constant or  $K_d$  ( $k_{\text{off}}/k_{\text{on}}$ ) of less than  $10^{-2} \text{ M}$ , less than  $5 \times 10^{-2} \text{ M}$ , less than  $10^{-3} \text{ M}$ , less than  $5 \times 10^{-3} \text{ M}$ , less than  $10^{-4} \text{ M}$ , less than  $5 \times 10^{-4} \text{ M}$ , less than  $10^{-5} \text{ M}$ , less than  $5 \times 10^{-5} \text{ M}$ , less than  $10^{-6} \text{ M}$ , less than  $5 \times 10^{-6} \text{ M}$ , less than  $10^{-7} \text{ M}$ , less than  $5 \times 10^{-7} \text{ M}$ , less than  $10^{-8} \text{ M}$ , less than  $5 \times 10^{-8} \text{ M}$ , less than  $10^{-9} \text{ M}$ , less than  $5 \times 10^{-9} \text{ M}$ , less than  $10^{-10} \text{ M}$ , less than  $5 \times 10^{-10} \text{ M}$ , less than  $10^{-11} \text{ M}$ , less than  $5 \times 10^{-11} \text{ M}$ , less than  $10^{-12} \text{ M}$ , less than  $5 \times 10^{-12} \text{ M}$ , less than  $10^{-13} \text{ M}$ , less than  $5 \times 10^{-13} \text{ M}$ , less than  $10^{-14} \text{ M}$ , less than  $5 \times 10^{-14} \text{ M}$ , less than  $10^{-15} \text{ M}$ , or less than  $5 \times 10^{-15} \text{ M}$ .

The present invention provides antibodies or fragment thereof that have a median effective concentration ( $\text{EC}_{50}$ ) of less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM, less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM, in an *in vitro* microneutralization assay. The median effective concentration is the concentration of antibody or antibody fragments that neutralizes 50% of the RSV in an *in vitro* microneutralization assay. In a preferred embodiment, antibody of the invention or fragment thereof has an  $\text{EC}_{50}$  of less than 0.01 nM, less than 0.025 nM, less than 0.05 nM, less than 0.1 nM, less than 0.25 nM, less than 0.5 nM, less than 0.75 nM, less than 1 nM,

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less than 1.25 nM, less than 1.5 nM, less than 1.75 nM, or less than 2 nM, in an *in vitro* microneutralization assay.

In a specific embodiment, an antibody of the present invention is SYNAGIS® or an antibody-binding fragment thereof (*e.g.*, one or more complementarity determining regions (CDRs) of SYNAGIS®). The amino acid sequence of SYNAGIS® is disclosed, *e.g.*, in Johnson et al., 1997, J. Infectious Disease 176:1215-1224, and U.S. Patent No. 5,824,307, each of which is incorporated herein by reference in its entirety. In alternative embodiment, an antibody of the present invention or fragment thereof is not SYNAGIS® or a fragment of SYNAGIS®, *i.e.*, is not an antibody comprising a VH domain of SEQ ID NO:7 and/or a VL domain of SEQ ID NO:8.

The present invention provides for antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising the amino acid sequence of SYNAGIS® with one or more amino acid residue substitutions in the variable light (VL) domain and/or variable heavy (VH) domain depicted in Figure 1. The present invention also provides for antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising the amino acid sequence of SYNAGIS® with one or more amino acid residue substitutions in one or more VL CDRs and/or one or more VH CDRs. In a specific embodiment, an antibody or fragment thereof comprises the amino acid sequence of SYNAGIS® with one or more amino acid residue substitutions of the amino acid residues indicated in bold face and underlining in Table 1. In accordance with this embodiment, the amino acid residue substitutions can be conservative or non-conservative. The antibody or antibody fragment generated by introducing substitutions in the VH domain, VH CDRs, VL domain and/or VL CDRs of SYNAGIS® can be tested *in vitro* and *in vivo*, for example, for its ability to bind to RSV F antigen, for its ability to neutralize RSV, or for its ability to prevent, treat or ameliorate one or more symptoms associated with a RSV infection.

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Table 1. CDR Sequences Of SYNAGIS®

CDR	Sequence	SEQ ID NO:
VH1	T <u>S</u> GMSVG	1
VH2	DIWWDD <u>DKKD</u> YNPSLK <u>S</u>	2
VH3	<u>S</u> MITN <u>W</u> YFDV	3
VL1	<u>K</u> COLSVGYMH	4
VL2	DTS <u>K</u> LAS	5
VL3	FQGS <u>G</u> YP <u>F</u> T	6

Bold faced & underlined amino acid residues are preferred residues which should be substituted.

5 In a specific embodiment, an antibody of the present invention is SYNAGIS®, AFFF, Pfl2, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8c7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S. Preferably, an antibody of the present invention is AFFF, Pfl2, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8c7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S. In another embodiment, an antibody is a Fab  
10 fragment of SYNAGIS®, AFFF, Pfl2, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8c7, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

AFFF, Pfl2, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A8c7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, and A1h5  
15 comprise the framework region and constant regions of SYNAGIS®. A4B4, A4B4(1), A4B4L1FR-S28R, and A4B4-F52S comprise the framework region and constant regions of SYNAGIS® with the exception that there is a valine at position 103, numbered as shown in Figure 2A and a glutamine at position 112, numbered as shown in Figure 2B. In  
20 certain embodiments, A4B4, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S comprise the framework region and constant regions of SYNAGIS®.

In another embodiment, the present invention provides for an antigen-binding fragment of AFFF, Pfl2, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8c7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S. In another embodiment, the present  
25 invention provides for one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising a VH chain and/or VL chain having the amino acid sequence of a VH chain and/or VL chain of AFFF, Pfl2, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8c7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1),  
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A4B4L1FR-S28R, or A4B4-F52S. In another embodiment, the present invention provides for one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising a VH domain and/or VL domain having the amino acid sequence of a VH domain and/or VL domain of AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8c7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S. In another embodiment, the present invention provides for antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising one or more CDRs having the amino acid sequence of one or more CDRs of AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8c7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S. In yet another embodiment, the present invention provides for one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising a combination of VH CDRs and/or VL CDRs having the amino acid sequence of VH CDRs and/or VL CDRs of AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8c7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, and/or A4B4-F52S.

20       The present invention provides antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising a variable heavy ("VH") chain having an amino acid sequence of any one of the VL domains listed in Table 2. The present invention also provides antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said

25 antibodies or antibody fragments comprising a VH domain having an amino acid sequence of any one of the VH domains listed in Table 2. In certain embodiments of the invention, an antibody or fragment thereof comprising a VH domain having an amino acid of any of one of the VH domains listed in Table 2 is not SYNAGIS®. The present invention also provides antibodies or fragments thereof that immunospecifically bind to one or more RSV

30 antigens, said antibodies or fragments comprising a VH complementarity determining region ("CDR") having an amino acid sequence of any one of the VH CDRs listed in Table 2 and/or Table 3. In certain embodiments of the invention, an antibody or fragment thereof comprising a VH CDR having an amino acid of any of one of the VH CDRs listed in Table 2 and/or Table 3 is not SYNAGIS®.

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Table 2. Antibodies &amp; Fragments Thereof

Antibody Name	VH Chain	VH Domain	VH CDR1	VH CDR2	VH CDR3	VL Chain	VL Domain	VL CDR1	VL CDR2	VL CDR3
**SYNAGIS	SEQ ID NO: 208	SEQ ID NO: 7	TSGMSVG (SEQ ID NO: 1)	DIWWDKKDY NPSLKS (SEQ ID NO: 2)	SMITNWFYFDV (SEQ ID NO: 3)	SEQ ID NO: 209	SEQ ID NO: 8	KCQLSVGYM H (SEQ ID NO: 4)	DTSKLAS (SEQ ID NO: 5)	FQSGGYPFT (SEQ ID NO: 6)
***AFF	SEQ ID NO: 210	SEQ ID NO: 9	TAGMSVG (SEQ ID NO: 10)	DIWWDKKDYN PSLKS (SEQ ID NO: 2)	SMITNWFYFDV (SEQ ID NO: 12)	SEQ ID NO: 211	SEQ ID NO: 13	SASSSVGYMH (SEQ ID NO: 14)	DTEKLAS (SEQ ID NO: 15)	FQSGGYPFT (SEQ ID NO: 16)
***P12f2	SEQ ID NO: 212	SEQ ID NO: 17	TPGMSVG (SEQ ID NO: 18)	DIWWDKKHYN PSLKD (SEQ ID NO: 19)	DMIFNWFYFDV (SEQ ID NO: 20)	SEQ ID NO: 213	SEQ ID NO: 21	SLSSRVGYMH (SEQ ID NO: 22)	DTEFLSS (SEQ ID NO: 23)	FQSGGYPFT (SEQ ID NO: 24)
***P12f4	SEQ ID NO: 214	SEQ ID NO: 24	TPGMSVG (SEQ ID NO: 18)	DIWWDGKKHYN PSLKD (SEQ ID NO: 25)	DMIFNWFYFDV (SEQ ID NO: 20)	SEQ ID NO: 215	SEQ ID NO: 26	SLSSRVGYMH (SEQ ID NO: 22)	DTRGLPS (SEQ ID NO: 27)	FQSGGYPFT (SEQ ID NO: 28)
***P11d4	SEQ ID NO: 216	SEQ ID NO: 28	TPGMSVG (SEQ ID NO: 18)	DIWWDGKKHYN PSLKD (SEQ ID NO: 25)	DMIFNWFYFDV (SEQ ID NO: 29)	SEQ ID NO: 217	SEQ ID NO: 30	SPSSRVGYMH (SEQ ID NO: 31)	DTMRLAS (SEQ ID NO: 32)	FQSGGYPFT (SEQ ID NO: 33)
***Ale9	SEQ ID NO: 218	SEQ ID NO: 33	TAGMSVG (SEQ ID NO: 10)	DIWWDGKKHYN PSLKD (SEQ ID NO: 25)	DMIFNWFYFDV (SEQ ID NO: 29)	SEQ ID NO: 219	SEQ ID NO: 34	SLSSRVGYMH (SEQ ID NO: 22)	DTEKLSS (SEQ ID NO: 35)	FQSGGYPFT (SEQ ID NO: 36)
***A12a6	SEQ ID NO: 220	SEQ ID NO: 36	TAGMSVG (SEQ ID NO: 10)	DIWWDGKKDYN PSLKD (SEQ ID NO: 37)	DMIFNWFYFDV (SEQ ID NO: 20)	SEQ ID NO: 221	SEQ ID NO: 38	SASSRVGYMH (SEQ ID NO: 39)	DTEKLSS (SEQ ID NO: 40)	FQSGGYPFT (SEQ ID NO: 41)

***A13c4	SEQ ID NO: 222	SEQ ID NO:40	TAGMSVG (SEQ ID NO:10)	DIWWDGKKSYN PSLKD (SEQ ID NO:41)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO: 223	SEQ ID NO:42	SLSSRVGYMH (SEQ ID NO:22)	DTMYOSS (SEQ ID NO:43)	FQSGYPFT (SEQ ID NO:6)
***A17d4	SEQ ID NO: 224	SEQ ID NO:44	TAGMSVG (SEQ ID NO:10)	DIWWDGKKSY NPSLKD (SEQ ID NO:45)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO: 225	SEQ ID NO:46	LPSSRVGYM H (SEQ ID NO:47)	DTMYOSS (SEQ ID NO:43)	FQSGYPFT (SEQ ID NO:6)
***A4B4	SEQ ID NO: 226	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDGKKH YNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO: 227	SEQ ID NO:49	SASSRVGYM H (SEQ ID NO:39)	DTFFLDS (SEQ ID NO:50)	FQSGYPFT (SEQ ID NO:6)
***A8c7	SEQ ID NO: 228	SEQ ID NO:51	TAGMSVG (SEQ ID NO:10)	DIWWDGKKSY NPSLKD (SEQ ID NO:45)	DMIFNWFYD V (SEQ ID NO:29)	SEQ ID NO: 229	SEQ ID NO:52	SPSSRVGYM H (SEQ ID NO:31)	DTRYOSS (SEQ ID NO:53)	FQSGYPFT (SEQ ID NO:6)
*1X- 493LIFR	SEQ ID NO: 230	SEQ ID NO:7	TSGMSVG (SEQ ID NO:1)	DIWWDGKKDYN PSLKS (SEQ ID NO:2)	SMITNWFYD (SEQ ID NO:3)	SEQ ID NO: 231	SEQ ID NO:54	SASSSVGYMH (SEQ ID NO:14)	DTSKLAS (SEQ ID NO:5)	FQSGYPFT (SEQ ID NO:6)
*H3-3F4	SEQ ID NO: 232	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDGKKDYN PSLKS (SEQ ID NO:2)	DMIFNWFYD (SEQ ID NO:29)	SEQ ID NO: 233	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTFKLAS (SEQ ID NO:15)	FQSGYPFT (SEQ ID NO:6)
*M3H9	SEQ ID NO: 234	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDGKKDYN PSLKS (SEQ ID NO:2)	DMIFNWFYD (SEQ ID NO:29)	SEQ ID NO: 235	SEQ ID NO:70	SASSSVGYMH (SEQ ID NO:14)	DTYKQTS (SEQ ID NO:57)	FQSGYPFT (SEQ ID NO:6)
*Y10H6	SEQ ID NO: 236	SEQ ID NO:55	TAGMSVG (SEQ ID NO:10)	DIWWDGKKDYN PSLKS (SEQ ID NO:2)	DMIFNWFYD (SEQ ID NO:29)	SEQ ID NO: 237	SEQ ID NO:58	SASSSVGYMH (SEQ ID NO:14)	DTRYLSS (SEQ ID NO:59)	FQSGYPFT (SEQ ID NO:6)



*DG	SEQ ID NO: 238	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDKKDYN PSLKS (SEQ ID NO:2)	DMITN <del>F</del> YFDV (SEQ ID NO:79)	SEQ ID NO: 239	SEQ ID NO:56	SASSSVGYMH (SEQ ID NO:14)	DTEK <del>L</del> AS (SEQ ID NO:15)	FQSGYPFT (SEQ ID NO:6)
AFF(1)	SEQ ID NO: 240	SEQ ID NO:9	TAGMSVG (SEQ ID NO:10)	DIWWDKKDYN PSLKS (SEQ ID NO:2)	SMTN <del>F</del> YFDV (SEQ ID NO:12)	SEQ ID NO: 241	SEQ ID NO:60	SASSSVGYMH (SEQ ID NO:14)	DTEK <del>L</del> AS (SEQ ID NO:15)	FQSGYPFT (SEQ ID NO:61)
*6H8	SEQ ID NO: 242	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDKKDYN PSLKS (SEQ ID NO:2)	DMITN <del>F</del> YFDV (SEQ ID NO:79)	SEQ ID NO: 243	SEQ ID NO:62	SASSSVGYMH (SEQ ID NO:14)	DTEK <del>L</del> TS (SEQ ID NO:63)	FQSGYPFT (SEQ ID NO:6)
*L1-7E5	SEQ ID NO: 244	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDKKDYN PSLKS (SEQ ID NO:2)	DMITN <del>F</del> YFDV (SEQ ID NO:79)	SEQ ID NO: 245	SEQ ID NO:64	SASSSVGYMH (SEQ ID NO:39)	DTEK <del>L</del> AS (SEQ ID NO:15)	FQSGYPFT (SEQ ID NO:6)
*L2-15B10	SEQ ID NO: 246	SEQ ID NO:78	TAGMSVG (SEQ ID NO:10)	DIWWDKKDYN PSLKS (SEQ ID NO:2)	DMITN <del>F</del> YFDV (SEQ ID NO:79)	SEQ ID NO: 247	SEQ ID NO:65	SASSSVGYMH (SEQ ID NO:14)	DTE <del>R</del> LAS (SEQ ID NO:66)	FQSGYPFT (SEQ ID NO:6)
*A13a11	SEQ ID NO: 248	SEQ ID NO:67	TAGMSVG (SEQ ID NO:10)	DIWWDKK <del>H</del> YN PSLKD (SEQ ID NO:19)	DMITN <del>W</del> YFDV (SEQ ID NO:29)	SEQ ID NO: 249	SEQ ID NO:68	SPSSRVGYMH (SEQ ID NO:31)	DTY <del>R</del> HSS (SEQ ID NO:69)	FQSGYPFT (SEQ ID NO:6)
*A1h5	SEQ ID NO: 250	SEQ ID NO:33	TAGMSVG (SEQ ID NO:10)	DIWWDGKK <del>H</del> YN PSLKD (SEQ ID NO:25)	DMITN <del>W</del> YFDV (SEQ ID NO:29)	SEQ ID NO: 251	SEQ ID NO:71	SLSSSVGYMH (SEQ ID NO:72)	DTE <del>F</del> HRS (SEQ ID NO:73)	FQSGYPFT (SEQ ID NO:6)
A4B4(1)	SEQ ID NO: 252	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDKK <del>H</del> YNPSLKD (SEQ ID NO:19)	DMITN <del>F</del> YFDV (SEQ ID NO:20)	SEQ ID NO: 253	SEQ ID NO:74	SASSRVGYM H (SEQ ID NO:39)	DTLL <del>L</del> DS (SEQ ID NO:75)	FQSGYPFT (SEQ ID NO:6)

***A4B41.1 FR-S28R	SEQ ID NO: 254	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDKKH YNPSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO: 255	SEQ ID NO:11	SASSRVGYM H (SEQ ID NO:39)	DTSKLAS (SEQ ID NO:5)	FQSGGYPFT (SEQ ID NO:6)
***A4B4- F52S	SEQ ID NO: 256	SEQ ID NO:48	TAGMSVG (SEQ ID NO:10)	DIWWDKKHYN PSLKD (SEQ ID NO:19)	DMIFNFYFDV (SEQ ID NO:20)	SEQ ID NO: 257	SEQ ID NO:76	SASSRVGYMH (SEQ ID NO:39)	DTSFLDS (SEQ ID NO:77)	FQSGGYPFT (SEQ ID NO:6)

Bold faced & underlined amino acid residues are the residues which differ from the amino acid sequence in SYNAGIS®; Fab fragment produced (\*); Monoclonal antibody produced (\*\*); Fab fragment & monoclonal antibody produced (\*\*\*)

Table 3. CDR Sequences

VH CDR1	VH CDR2	VH CDR3	VL CDR1	VL CDR2	VL CDR3
TSGMSVG (SEQ ID NO:1)	DIWWD <del>D</del> KK <del>D</del> YNPSLK <del>S</del> (SEQ ID NO:2)	<del>S</del> MTN <del>W</del> YFDV (SEQ ID NO:3)	KCOLSVGYMH (SEQ ID NO:4)	DTSK <del>I</del> AS (SEQ ID NO:5)	FQSGYPFT (SEQ ID NO:6)
TPGMSVG (SEQ ID NO:18)	DIWWD <del>D</del> KK <del>H</del> YNPSLK <del>D</del> (SEQ ID NO:19)	<del>D</del> MTN <del>F</del> YFDV (SEQ ID NO:29)	KCOSSVGYMH (SEQ ID NO:80)	DTSYL <del>A</del> S (SEQ ID NO:81)	FQFSYPFT (SEQ ID NO:6)
TAGMSVG (SEQ ID NO:10)	DIWWD <del>D</del> KK <del>H</del> YNPSLK <del>S</del> (SEQ ID NO:82)	<del>D</del> MTN <del>W</del> YFDV (SEQ ID NO:83)	KCOSRVGYMH (SEQ ID NO:84)	DTSYL <del>S</del> S (SEQ ID NO:85)	FQGSYPFT (SEQ ID NO:61)
	DIWWD <del>D</del> KK <del>D</del> YNPSLK <del>D</del> (SEQ ID NO:86)	<del>D</del> MTN <del>W</del> YFDV (SEQ ID NO:29)	KCOLRVGYMH (SEQ ID NO:87)	D <del>T</del> K <del>K</del> L <del>S</del> S (SEQ ID NO:88)	
	DIWWD <del>D</del> KK <del>H</del> YNPSLK <del>S</del> (SEQ ID NO:91)	<del>D</del> MTN <del>F</del> YFDV (SEQ ID NO:20)	KL <del>O</del> LSVGYMH (SEQ ID NO:89)	D <del>T</del> F <del>V</del> L <del>S</del> S (SEQ ID NO:90)	
	DIWWD <del>D</del> KK <del>D</del> YNPSLK <del>D</del> (SEQ ID NO:93)	<del>S</del> MTN <del>F</del> YFDV (SEQ ID NO:12)	KLOSSVGYMH (SEQ ID NO:92)	D <del>T</del> F <del>K</del> L <del>A</del> S (SEQ ID NO:15)	
	DIWWD <del>G</del> KK <del>H</del> YNPSLK <del>D</del> (SEQ ID NO:25)	<del>S</del> MTN <del>W</del> YFDV (SEQ ID NO:94)	KLOS <del>R</del> VGYMH (SEQ ID NO:95)	D <del>T</del> F <del>K</del> L <del>S</del> S (SEQ ID NO:96)	
	DIWWD <del>G</del> KK <del>D</del> YNPSLK <del>S</del> (SEQ ID NO:100)	<del>S</del> MTN <del>F</del> YFDV (SEQ ID NO:97)	KL <del>O</del> L <del>R</del> VGYMH (SEQ ID NO:98)	D <del>T</del> F <del>V</del> L <del>A</del> S (SEQ ID NO:99)	
	DIWWD <del>G</del> KK <del>D</del> YNPSLK <del>D</del> (SEQ ID NO:103)		KL <del>S</del> L <del>S</del> VGYMH (SEQ ID NO:101)	D <del>T</del> S <del>K</del> L <del>P</del> S (SEQ ID NO:102)	
	DIWWD <del>G</del> KK <del>H</del> YNPSLK <del>S</del> (SEQ ID NO:106)		KL <del>S</del> SSVGYMH (SEQ ID NO:104)	D <del>T</del> S <del>G</del> L <del>A</del> S (SEQ ID NO:105)	
	DIWWD <del>D</del> KK <del>S</del> YNPSLK <del>S</del> (SEQ ID NO:109)		KL <del>S</del> SRVGYMH **(SEQ ID NO:107)	D <del>T</del> S <del>G</del> L <del>P</del> S (SEQ ID NO:108)	

	DIWWDGKKSYNPSLKD (SEQ ID NO:111)		KLSLRVGVMH (SEQ ID NO:110)	DTRGLPS (SEQ ID NO:27)	
	DIWWDGKKSYNPSLKS (SEQ ID NO:114)		KCSLSVGVMH (SEQ ID NO:112)	DTRKLAS (SEQ ID NO:113)	
	DIWWDGKKSYNPSLKD (SEQ ID NO:41)		KCSSSVGVMH (SEQ ID NO:115)	DTRGLAS (SEQ ID NO:116)	
			KCSSRVGVMH (SEQ ID NO:117)	DTRKLP (SEQ ID NO:118)	
			KCSLRVGVMH (SEQ ID NO:119)	DTMRLAS (SEQ ID NO:32)	
			SLSLSVGVMH (SEQ ID NO:120)	DTMKLAS (SEQ ID NO:121)	
			SLSSSVGVMH (SEQ ID NO:122)	DTSRLAS (SEQ ID NO:123)	
			SLSSRVGVMH (SEQ ID NO:22)	DTSLLAS (SEQ ID NO:124)	
			SLSLRVGVMH (SEQ ID NO:125)	DTSLIDS (SEQ ID NO:126)	
			SCOLSVGVMH (SEQ ID NO:127)	DTSKLDS (SEQ ID NO:128)	
			SCOSSVGVMH (SEQ ID NO:129)	DTLLIDS (SEQ ID NO:75)	
			SCOSRVGVMH (SEQ ID NO:130)	DTLKLDS (SEQ ID NO:131)	

			<u>SCQLRVGYMH</u> (SEQ ID NO:132)	<u>DTLLAS</u> (SEQ ID NO:133)	
			<u>SLQLSVGYMH</u> (SEQ ID NO:134)	<u>DTLKLAS</u> (SEQ ID NO:135)	
			<u>SLOSSVGYMH</u> (SEQ ID NO:136)	<u>DTSKLSS</u> (SEQ ID NO:137)	
			<u>SLQSRVGYMH</u> (SEQ ID NO:138)	<u>DTSKOAS</u> (SEQ ID NO:139)	
			<u>SLQLRVGYMH</u> (SEQ ID NO:140)	<u>DTSKOSS</u> (SEQ ID NO:141)	
			<u>SCSLSVGYMH</u> (SEQ ID NO:142)	<u>DTSYLAS</u> (SEQ ID NO:143)	
			<u>SCSSSVGYMH</u> (SEQ ID NO:144)	<u>DTSYLSS</u> (SEQ ID NO:145)	
			<u>SCSSRVGYMH</u> (SEQ ID NO:146)	<u>DTSYQAS</u> (SEQ ID NO:147)	
			<u>SCSLRVGYMH</u> (SEQ ID NO:148)	<u>DTSYQSS</u> (SEQ ID NO:149)	
			<u>KPSSRVGYMH</u> (SEQ ID NO:150)	<u>DTMYQAS</u> (SEQ ID NO:151)	
			<u>KPSLRVGYMH</u> (SEQ ID NO:152)	<u>DTMYQSS</u> (SEQ ID NO:153)	
			<u>KPSSSVGYMH</u> (SEQ ID NO:153)	<u>DTMKQAS</u> (SEQ ID NO:154)	

			<u>KPSLS</u> VG <del>Y</del> MH (SEQ ID NO:155)	D <del>T</del> M <del>K</del> O <del>S</del> S (SEQ ID NO:156)	
			<u>KPOS</u> R <del>V</del> G <del>Y</del> MH (SEQ ID NO:157)	D <del>T</del> M <del>Y</del> L <del>A</del> S (SEQ ID NO:158)	
			<u>KPOL</u> R <del>V</del> G <del>Y</del> MH (SEQ ID NO:159)	D <del>T</del> M <del>Y</del> L <del>S</del> S (SEQ ID NO:160)	
			<u>KPOSS</u> V <del>G</del> YMH (SEQ ID NO:161)	D <del>T</del> M <del>K</del> L <del>A</del> S (SEQ ID NO:162)	
			<u>KPOL</u> S <del>V</del> G <del>Y</del> MH (SEQ ID NO:163)	D <del>T</del> M <del>K</del> L <del>S</del> S (SEQ ID NO:164)	
			<u>SPSS</u> R <del>V</del> G <del>Y</del> MH (SEQ ID NO:31)	D <del>T</del> S <del>K</del> L <del>S</del> S (SEQ ID NO:165)	
			<u>SPSL</u> R <del>V</del> G <del>Y</del> MH (SEQ ID NO:166)	D <del>T</del> R <del>Y</del> O <del>A</del> S (SEQ ID NO:167)	
			<u>SPSSS</u> V <del>G</del> YMH (SEQ ID NO:168)	D <del>T</del> R <del>Y</del> O <del>S</del> S (SEQ ID NO:53)	
			<u>SPSL</u> S <del>V</del> G <del>Y</del> MH (SEQ ID NO:169)	D <del>T</del> R <del>K</del> O <del>A</del> S (SEQ ID NO:170)	
			<u>SPOS</u> R <del>V</del> G <del>Y</del> MH (SEQ ID NO:171)	D <del>T</del> R <del>K</del> O <del>S</del> S (SEQ ID NO:172)	
			<u>SPOL</u> R <del>V</del> G <del>Y</del> MH (SEQ ID NO:173)	D <del>T</del> R <del>K</del> L <del>A</del> S (SEQ ID NO:174)	
			<u>SPOSS</u> V <del>G</del> YMH (SEQ ID NO:175)	D <del>T</del> R <del>K</del> L <del>S</del> S (SEQ ID NO:176)	

			<u>SPQLSVGYMH</u> (SEQ ID NO:177)	<u>DTRYLAS</u> (SEQ ID NO:178)	
			<u>KAQSRVGYMH</u> (SEQ ID NO:179)	<u>DTRYLSS</u> (SEQ ID NO:59)	
			<u>KAOLRVGYMH</u> (SEQ ID NO:180)	<u>DTEFLDS</u> (SEQ ID NO:50)	
			<u>KAQSSVGYMH</u> (SEQ ID NO:181)	<u>DTSFLDS</u> (SEQ ID NO:77)	
			<u>KAOLSVGYMH</u> (SEQ ID NO:182)		
			<u>KASSRVGYMH</u> (SEQ ID NO:183)		
			<u>KASLRVGYMH</u> (SEQ ID NO:184)		
			<u>KASSSVGYMH</u> (SEQ ID NO:185)		
			<u>KASLSVGYMH</u> (SEQ ID NO:186)		
			<u>SASSRVGYMH</u> (SEQ ID NO:39)		
			<u>SASLRVGYMH</u> (SEQ ID NO:187)		
			<u>SASSSVGYMH</u> (SEQ ID NO:14)		

				<b><u>SASL</u>SVGYMH</b> (SEQ ID NO:188)			
				<b><u>SAQSR</u>VGVMH</b> (SEQ ID NO:189)			
				<b><u>SAQLR</u>VGVMH</b> (SEQ ID NO:190)			
				<b><u>SAOSS</u>VGVMH</b> (SEQ ID NO:191)			
				<b><u>LPSSR</u>VGVMH</b> (SEQ ID NO:47)			
				<b><u>LPSSL</u>VGVMH</b> (SEQ ID NO:192)			
				<b><u>LPSSS</u>VGVMH</b> (SEQ ID NO:193)			
				<b><u>LPSLR</u>VGVMH</b> (SEQ ID NO:194)			
				<b><u>LCSSR</u>VGVMH</b> (SEQ ID NO:195)			
				<b><u>LCSSL</u>VGVMH</b> (SEQ ID NO:196)			
				<b><u>LCSSS</u>VGVMH</b> (SEQ ID NO:197)			
				<b><u>LCSLR</u>VGVMH</b> (SEQ ID NO:198)			



				<b><u>LPQSR</u></b> VG <sup>TM</sup> H (SEQ ID NO:199)			
				<b><u>LPQLS</u></b> VG <sup>TM</sup> H (SEQ ID NO:200)			
				<b><u>LPQSS</u></b> VG <sup>TM</sup> H (SEQ ID NO:201)			
				<b><u>LPQLR</u></b> VG <sup>TM</sup> H (SEQ ID NO:202)			
				<b><u>LCQSR</u></b> VG <sup>TM</sup> H (SEQ ID NO:203)			
				<b><u>LCQLS</u></b> VG <sup>TM</sup> H (SEQ ID NO:204)			
				<b><u>LCOSS</u></b> VG <sup>TM</sup> H (SEQ ID NO:205)			
				<b><u>LCQLR</u></b> VG <sup>TM</sup> H (SEQ ID NO:206)			
				<b><u>SAQLS</u></b> VG <sup>TM</sup> H (SEQ ID NO:207)			

In one embodiment of the present invention, antibodies or fragments thereof comprise a VH CDR1 having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:10 or SEQ ID NO:18. In another embodiment, antibodies or fragments thereof comprise a VH CDR2 having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:37, SEQ ID NO:41 or SEQ ID NO:45. In another embodiment, antibodies comprise a VH CDR3 having the amino acid sequence of SEQ ID NO:3, SEQ ID NO:12, SEQ ID NO:20, SEQ ID NO:29 or SEQ ID NO:79. In a preferred embodiment, antibodies or fragments thereof comprise a VH CDR1 having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:10 or SEQ ID NO:18, a VH CDR2 having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:37 or SEQ ID NO:41, SEQ ID NO:45, and a VH CDR3 having the amino acid sequence of SEQ ID NO:3, SEQ ID NO:12, SEQ ID NO:20, SEQ ID NO:29 or SEQ ID NO:79.

The present invention also provides antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising a variable light ("VL") domain having an amino acid sequence of any one of the VL domains listed in Table 2. The present invention also provides antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or fragments comprising a VL CDR having an amino acid sequence of any one of the VL CDRs listed in Table 2 and/or Table 3.

In one embodiment of the present invention, antibodies or fragments thereof comprise a VL CDR1 having the amino acid sequence of SEQ ID NO:4, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:31, SEQ ID NO:39, or SEQ ID NO:47. In another embodiment, antibodies or fragments thereof comprise a VL CDR2 having the amino acid sequence of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:73, SEQ ID NO:75 or SEQ ID NO:77. In another embodiment, antibodies or fragments thereof comprise a VL CDR3 having the amino acid sequence of SEQ ID NO:6, SEQ ID NO:16 or SEQ ID NO:61. In a preferred embodiment, antibodies or fragments thereof comprise a VL CDR1 having the amino acid sequence of SEQ ID NO:4, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:31, SEQ ID NO:39, or SEQ ID NO:47, a VL CDR2 having the amino acid sequence of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:73, SEQ ID

NO:75 or SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6, SEQ ID NO:16 or SEQ ID NO:61.

The present invention also provides antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising a VH domain disclosed herein combined with a VL domain disclosed herein, or other VL domain. The present invention further provides antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens, said antibodies or fragments comprising a VL domain disclosed herein combined with a VH domain disclosed herein, or other VH domain. In a preferred embodiment, antibodies or fragments thereof that immunospecifically bind to a RSV antigen comprise a VH domain having the amino acid sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:17, SEQ ID NO:24, SEQ ID NO:28, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:40, SEQ ID NO:44, SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:55, SEQ ID NO:67 or SEQ ID NO:78 and a VL domain having the amino acid sequence of SEQ ID NO:8, SEQ ID NO:13, SEQ ID NO:21, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:34, SEQ ID NO:38, SEQ ID NO:42, SEQ ID NO:46, SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:74 or SEQ ID NO:76.

The present invention also provides antibodies or fragments thereof comprising one or more VH CDRs and one or more VL CDRs listed in Table 2 and/or Table 3. In particular, the invention provides for an antibody or fragment thereof comprising a VH CDR1 and a VL CDR1, a VH CDR1 and a VL CDR2, a VH CDR1 and a VL CDR3, a VH CDR2 and a VL CDR1, VH CDR2 and VL CDR2, a VH CDR2 and a VL CDR3, a VH CDR3 and a VH CDR1, a VH CDR3 and a VL CDR2, a VH CDR3 and a VL CDR3, or any combination thereof of the VH CDRs and VL CDRs listed in Table 2. The invention also provides for an antibody or fragment thereof comprising a VH CDR1 and a VL CDR1, a VH CDR1 and a VL CDR2, a VH CDR1 and a VL CDR3, a VH CDR2 and a VL CDR1, VH CDR2 and VL CDR2, a VH CDR2 and a VL CDR3, a VH CDR3 and a VH CDR1, a VH CDR3 and a VL CDR2, a VH CDR3 and a VL CDR3, or any combination thereof of the VH CDRs and VL CDRs listed in Table 3. The invention also provides for an antibody or fragment thereof comprising a VH CDR1 and a VL CDR1, a VH CDR1 and a VL CDR2, a VH CDR1 and a VL CDR3, a VH CDR2 and a VL CDR1, VH CDR2 and VL CDR2, a VH CDR2 and a VL CDR3, a VH CDR3 and a VH CDR1, a VH CDR3 and a VL CDR2, a VH CDR3 and a VL CDR3, or any combination thereof of the VH CDRs and VL CDRs listed in Table 2 and Table 3.

In one embodiment, an antibody or fragment thereof comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:10 or SEQ ID NO:18 and a VL CDR1 having the amino acid sequence of SEQ ID NO:4, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:31, SEQ ID NO:39, or SEQ ID NO:47. In another embodiment, an antibody of  
5 the present invention or fragment thereof comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:10 or SEQ ID NO:18 and a VL CDR2 having the amino acid sequence of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:73,  
10 SEQ ID NO:75, or SEQ ID NO:77. In another embodiment, an antibody of the present invention or fragment thereof comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:10 or SEQ ID NO:18 and a VL CDR3 having the amino acid sequence of SEQ ID NO:6, SEQ ID NO:16 or SEQ ID NO:61.

In another embodiment, an antibody of the present invention or fragment thereof  
15 comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:37, SEQ ID NO:41 or SEQ ID NO:45 and a VL CDR1 having the amino acid sequence of SEQ ID NO:4, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:31, SEQ ID NO:39 or SEQ ID NO:47. In another embodiment, an antibody of the present invention or fragment thereof comprises a VH CDR2 having the amino acid sequence of  
20 SEQ ID NO:2, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:37, SEQ ID NO:41 or SEQ ID NO:45 and a VL CDR2 having the amino acid sequence of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:73, SEQ ID NO:75, or SEQ ID NO:77. In another  
25 embodiment, an antibody of the present invention or fragment thereof comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:2, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:37, SEQ ID NO:41 or SEQ ID NO:45 and a VL CDR3 having the amino acid sequence of SEQ ID NO:6, SEQ ID NO:16, or SEQ ID NO:61.

In another embodiment, an antibody of the present invention or fragment thereof  
30 comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:3, SEQ ID NO:12, SEQ ID NO:20, SEQ ID NO:29 or SEQ ID NO:79 and a VL CDR1 having the amino acid sequence of SEQ ID NO:4, SEQ ID NO:14, SEQ ID NO:22, SEQ ID NO:31, SEQ ID NO:39 or SEQ ID NO:47. In another embodiment, an antibody of the present invention or fragment thereof comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:3,  
35 SEQ ID NO:12, SEQ ID NO:20, SEQ ID NO:29 or SEQ ID NO:79 and a VL CDR2 having

the amino acid sequence of SEQ ID NO:5, SEQ ID NO:15, SEQ ID NO:23, SEQ ID NO:27, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:43, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:63, SEQ ID NO:66, SEQ ID NO:69, SEQ ID NO:73, SEQ ID NO:75, or SEQ ID NO:77. In a preferred embodiment, an antibody of the present invention or fragment thereof comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:3, SEQ ID NO:12, SEQ ID NO:20, SEQ ID NO:29 or SEQ ID NO:79 and a VL CDR3 having the amino acid sequence of SEQ ID NO:6, SEQ ID NO:16, or SEQ ID NO:61.

The present invention also provides for nucleic acid molecules, generally isolated, encoding an antibody of the invention or fragment thereof. In a specific embodiment, isolated nucleic acid molecules of the invention encode for SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S. Preferably, isolated nucleic acid molecules of the invention encode for AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S. In another embodiment, isolated nucleic acid molecules of the invention encode for an antigen-binding fragment of SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

In another embodiment, an isolated nucleic acid molecule(s) of the invention encodes an antibody or fragment thereof comprising a VH domain having an amino acid sequence of any one of the VH domains listed in Table 2. In another embodiment, an isolated nucleic acid molecule(s) of the invention encodes an antibody or fragment thereof comprising a VH CDR1 having an amino acid sequence of any one of the VH CDR1s listed in Table 2 or Table 3. In another embodiment, an isolated nucleic acid molecule(s) of the invention encodes an antibody or fragment thereof comprising a VH CDR2 having an amino acid sequence of any one of the VH CDR2s listed in Table 2 or Table 3. In yet another embodiment, an isolated nucleic acid molecule(s) of the invention encodes an antibody or fragment thereof comprising a VH CDR3 having an amino acid sequence of any one of the VH CDR3s listed in Table 2 or Table 3.

In another embodiment, an isolated nucleic acid molecule(s) of the invention encodes an antibody or fragment thereof comprising a VL domain having an amino acid sequence of any one of the VL domains listed in Table 2. In another embodiment, an

isolated nucleic acid molecule(s) of the present invention encodes an antibody or fragment thereof comprising a VL CDR1 having amino acid sequence of any one of the VL CDR1s listed in Table 2 or Table 3. In another embodiment, an isolated nucleic acid molecule(s) of the present invention encodes an antibody or fragment thereof comprising a VL CDR2  
5 having an amino acid sequence of any one of the VL CDR2s listed in Table 2 or Table 3. In yet another embodiment, an isolated nucleic acid molecule(s) of the present invention encodes an antibody or fragment thereof comprising a VL CDR3 having an amino acid sequence of any one of the VL CDR3s listed in Table 2 or Table 3.

In another embodiment, a nucleic acid molecule(s) of the invention encodes an  
10 antibody or fragment thereof comprising a VH domain having an amino acid sequence of any one of the VH domains listed in Table 2 and a VL domain having an amino acid sequence of any one of the VL domains listed in Table 2. In another embodiment, a nucleic acid molecule(s) of the invention encodes an antibody or fragment thereof comprising a VH CDR1, a VL CDR1, a VH CDR2, a VL CDR2, a VH CDR3, a VL CDR3, or any  
15 combination thereof having an amino acid sequence listed in Table 2. In another embodiment, a nucleic acid molecule(s) of the invention encodes an antibody or fragment thereof comprising a VH CDR1, a VL CDR1, a VH CDR2, a VL CDR2, a VH CDR3, a VL CDR3, or any combination thereof having an amino acid sequence listed in Table 3. In another embodiment, a nucleic acid molecule(s) of the invention encodes an antibody or  
20 fragment thereof comprising a VH CDR1, a VL CDR1, a VH CDR2, a VL CDR2, a VH CDR3, a VL CDR3, or any combination thereof having an amino acid sequence listed in Table 2 and Table 3.

The present invention also provides antibodies or fragments thereof comprising derivatives of the VH domains, VH CDRs, VL domains, and VL CDRs described herein  
25 that immunospecifically bind to an RSV antigen. The present invention also provides antibodies or fragments thereof comprising derivatives of SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S, wherein said antibodies or fragments thereof immunospecifically bind to one  
30 or more RSV antigens. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which results in amino acid substitutions. Preferably, the derivatives include less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid  
35 substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions,

less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the original molecule. In a preferred embodiment, the derivatives have conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which  
5 the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine,  
10 cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for  
15 biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed and the activity of the protein can be determined.

In a specific embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises a nucleotide sequence that hybridizes to the nucleotide sequence(s) encoding SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4,  
20 A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 °C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in  
25 6xSSC at about 45 °C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3).

30 In another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4,

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A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

In a specific embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence of a VH domain or an amino acid sequence a VL domain encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding any one of the VH or VL domains listed in Table 2 under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 °C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds. , 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3). In another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence of a VH CDR or an amino acid sequence of a VL CDRs encoded by a nucleotide sequence that hybridizes to the nucleotide sequence encoding any one of the VH CDRs or VL CDRs listed in Table 2 or Table 3 under stringent conditions *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 °C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art. In yet another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence of a VH CDR and an amino acid sequence of a VL CDR encoded by nucleotide sequences that hybridizes to the nucleotide sequences encoding any one of the VH CDRs and VL CDRs, respectively, listed in Table 2 or Table 3 under stringent conditions, *e.g.*, hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 °C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65 °C, under highly stringent conditions, *e.g.*, hybridization to filter-bound nucleic acid in 6xSSC at about 45 °C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68 °C, or under other stringent hybridization conditions which are known to those of skill in the art .

In another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence of a VH domain that is at least



35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to any one of the VH domains listed in Table 2. In another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence of one or more VH CDRs that are at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to any of the VH CDRs listed in Table 2 or Table 3.

In another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence of a VL domain that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to any one of the VL domains listed in Table 2. In another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence of one or more VL CDRs that are at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to any of the VL CDRs listed in Table 2 or Table 3.

In another embodiment, an antibody or fragment thereof that immunospecifically binds to a RSV antigen comprises an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to an amino acid sequence of SYNAGIS®, AFFE, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFE(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S

The present invention also encompasses antibodies or fragments thereof that compete with an antibody or Fab fragment listed in Table 2 for binding to a RSV antigen. In particular, the present invention encompasses antibodies or fragments thereof that compete with SYNAGIS® or an antigen-binding fragment thereof for binding to the RSV F glycoprotein. The present invention also encompasses VL domains, VH domains, VL CDRs, and VH CDRs that compete with a VL domain, VH domain, VL CDR, or VH CDR listed in Table 2 for binding to a RSV antigen. Further, the present invention encompasses VL CDRs and VL CDRs that compete with a VL CDR or VH CDR listed in Table 3 for binding to a RSV antigen.

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- The antibodies of the invention include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.
- 10 The present invention also provides antibodies of the invention or fragments thereof that comprise a framework region known to those of skill in the art. Preferably, the framework region of an antibody of the invention or fragment thereof is human. In a specific embodiment, an antibody of the invention or fragment thereof comprises the framework region of SYNAGIS®.
- 15 The present invention also provides antibodies of the invention or fragments thereof that comprise constant regions known to those of skill in the art. Preferably, the constant regions of an antibody of the invention or fragment thereof are human. In a specific embodiment, an antibody of the invention or fragment thereof comprises the constant regions of SYNAGIS®.
- 20 The present invention also provides for antibodies or fragments thereof that have half-lives in a mammal, preferably a human, of greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-lives of the antibodies of the present invention or fragments thereof in a mammal, preferably a human, results in a higher serum titer of said antibodies or antibody fragments in the mammal, and thus, reduces the frequency of the administration of said antibodies or antibody fragments and/or reduces the concentration of said antibodies or antibody fragments to be administered. Antibodies or fragments thereof having increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibodies or fragments thereof with increased *in vivo* half-lives can be generated by modifying (*e.g.*, substituting, deleting or adding) amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor (see, *e.g.*, PCT Publication No. WO 97/34631, which is incorporated herein by reference in its entirety). Such antibodies or fragments thereof can be tested for binding
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activity to RSV antigens as well as for *in vivo* efficacy using methods known to those skilled in the art, for example, by immunoassays described herein.

Further, antibodies or fragments thereof with increased *in vivo* half-lives can be generated by attaching to said antibodies or antibody fragments polymer molecules such as high molecular weight polyethyleneglycol (PEG). PEG can be attached to said antibodies or antibody fragments with or without a multifunctional linker either through site-specific conjugation of the PEG to the N- or C- terminus of said antibodies or antibody fragments or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation will be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by, *e.g.*, size exclusion or ion-exchange chromatography. PEG-derivatized antibodies or fragments thereof can be tested for binding activity to RSV antigens as well as for *in vivo* efficacy using methods known to those skilled in the art, for example, by immunoassays described herein.

The present invention also encompasses antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens, said antibodies or antibody fragments comprising the amino acid sequence of SYNAGIS® with mutations (*e.g.*, one or more amino acid substitutions) in the framework regions. In certain embodiments, antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens comprise the amino acid sequence of SYNAGIS® with one or more amino acid residue substitutions in the framework regions of the VH and/or VL domains depicted in Figure 1. In a specific embodiment, antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens comprise the framework regions depicted in Figure 2.

The present invention also encompasses antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens, said antibodies or fragments thereof comprising the amino acid sequence of SYNAGIS® with mutations (*e.g.*, one or more amino acid residue substitutions) in the variable and framework regions.

The present invention also provides for fusion proteins comprising an antibody or fragment thereof that immunospecifically binds to a RSV antigen and a heterologous polypeptide. Preferably, the heterologous polypeptide that the antibody or antibody fragment is fused to is useful for targeting the antibody to respiratory epithelial cells.

The present invention also provides for panels of antibodies or fragments thereof that immunospecifically bind to an RSV antigen. In specific embodiments, the invention

provides for panels of antibodies or fragments thereof having different affinities for an RSV antigen, different specificities for an RSV antigen, or different dissociation rates. The invention provides panels of at least 10, preferably at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, or at least 1000 antibodies or fragments thereof. Panels of antibodies can be used, for example, in 96 well plates for assays such as ELISAs.

The present invention further provides for compositions comprising one or more antibodies of the invention or fragments thereof. In a specific embodiment, a composition of the present invention comprises SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, and/or A4B4-F52S. In another specific embodiment, a composition of the present invention comprises an antigen-binding fragment of SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH domains having an amino acid sequence of any one of the VH domains listed in Table 2. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR1s having an amino acid sequence of any one of the VH CDR1s listed in Table 2 or Table 3. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR2s having an amino acid sequence of any one of the VH CDR2s listed in Table 2 or Table 3. In a preferred embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR3s having an amino acid sequence of any one of the VH CDR3s listed in Table 2 or Table 3.

In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VL domains having an amino acid sequence of any one of the VL domains listed in Table 2. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VL CDR1s having an amino acid sequence of any one of the VH CDR1s listed in Table 2 or Table 3. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more

VL CDR2s having an amino acid sequence of any one of the VL CDR2s listed in Table 2 or Table 3. In a preferred embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VL CDR3s having an amino acid sequence of any one of the VL CDR3s listed in Table 2 or Table 3.

5 In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH domains having an amino acid sequence of any one of the VH domains listed in Table 2 and one or more VL domains having an amino acid sequence of any one of the VL domains listed in Table 2. In another embodiment, a composition of the present invention comprises one or more antibodies or  
10 fragments thereof comprising one or more VH CDR1s having an amino acid sequence of any one of the VH CDR1s listed in Table 2 or Table 3 and one or more VL CDR1s having an amino acid sequence of any one of the VL CDR1s listed in Table 2 or Table 3. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR1s having an amino acid  
15 sequence of any one of the VH CDR1s listed in Table 2 or Table 3 and one or more VL CDR2s having an amino acid sequence of any one of the VL CDR2s listed in Table 2 or Table 3. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR1s having an amino acid sequence of any one of the VH CDR1s listed in Table 2 or Table 3 and one or more VL  
20 CDR3s having an amino acid sequence of any one of the VL CDR3s listed in Table 2 or Table 3.

In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR2s having an amino acid sequence of any one of the VH CDR2s listed in Table 2 or Table 3 and one or more VL  
25 CDR1s having an amino acid sequence of any one of the VL CDR1s listed in Table 2 or Table 3. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR2s having an amino acid sequence of any one of the VH CDR2s listed in Table 2 or Table 3 and one or more VL CDR2s having an amino acid sequence of any one of the VL CDR2s listed in Table 2 or  
30 Table 3. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR2s having an amino acid sequence of any one of the VH CDR2s listed in Table 2 or Table 3 and one or more VL CDR3s having an amino acid sequence of any one of the VL CDR3s listed in Table 2 or Table 3.

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In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR3s having an amino acid sequence of any one of the VH CDR3s listed in Table 2 or Table 3 and one or more VL CDR1s having an amino acid sequence of any one of the VL CDR1s listed in Table 2 or Table 3. In another embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR3s having an amino acid sequence of any one of the VH CDR3s listed in Table 2 or Table 3 and one or more VL CDR2s having an amino acid sequence of any one of the VL CDR2s listed in Table 2 or Table 3. In a preferred embodiment, a composition of the present invention comprises one or more antibodies or fragments thereof comprising one or more VH CDR3s having an amino acid sequence of any one of the VH CDR3s listed in Table 2 or Table 3 and one or more VL CDR3s having an amino acid sequence of any one of the VL CDR3s listed in Table 2 or Table 3. In yet another embodiment, a composition of the present invention comprises one or more fusion proteins of the invention.

As discussed in more detail below, a composition of the invention may be used either alone or in combination with other compositions. The antibodies or fragments thereof may further be recombinantly fused to a heterologous polypeptide at the N – or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, *e.g.*, PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

Antibodies of the present invention or fragments thereof may be used, for example, to purify, detect, and target RSV antigens, in both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies or fragments have use in immunoassays for qualitatively and quantitatively measuring levels of the RSV in biological samples such as sputum. See, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

#### 5.1.1. Antibody Conjugates

The present invention encompasses antibodies or fragments thereof recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a heterologous polypeptide (or portion thereof, preferably at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino

acids of the polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. For example, antibodies may be used to target heterologous polypeptides to particular cell types (*e.g.*, respiratory epithelial cells), either *in vitro* or *in vivo*, by fusing or conjugating the antibodies to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to heterologous polypeptides may also be used in *in vitro* immunoassays and purification methods using methods known in the art. See *e.g.*, PCT publication WO 93/21232; EP 439,095; Naramura *et al.*, Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies *et al.*, PNAS 89:1428-1432 (1992); and Fell *et al.*, J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entirety.

In one embodiment, a fusion protein of the invention comprises SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S and a heterologous polypeptide. In another embodiment, a fusion protein of the invention comprises an antigen-binding fragment of SYNAGIS®, AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S and a heterologous polypeptide. In another embodiment, a fusion protein of the invention comprises one or more VH domains having the amino acid sequence of any one of the VH domains listed in Table 2 or one or more VL domains having the amino acid sequence of any one of the VL domains listed in Table 2 and a heterologous polypeptide. In another embodiment, a fusion protein of the present invention comprises one or more VH CDRs having the amino acid sequence of any one of the VH CDRs listed in Table 2 or Table 3 and a heterologous polypeptide. In another embodiment, a fusion protein comprises one or more VL CDRs having the amino acid sequence of any one of the VL CDRs listed in Table 2 or Table 3 and a heterologous polypeptide. In another embodiment, a fusion protein of the invention comprises at least one VH domain and at least one VL domain listed in Table 2 and a heterologous polypeptide. In yet another embodiment, a fusion protein of the invention comprises at least one VH CDR and at least one VL CDR domain listed in Table 2 or Table 3 and a heterologous polypeptide.

The present invention further includes compositions comprising heterologous polypeptides fused or conjugated to antibody fragments. For example, the heterologous polypeptides may be fused or conjugated to a Fab fragment, Fd fragment, Fv fragment, F(ab)<sub>2</sub> fragment, or portion thereof. Methods for fusing or conjugating polypeptides to antibody portions are known in the art. See, *e.g.*, U.S. Patent Nos. 5,336,603, 5,622,929,

5,359,046, 5,349,053, 5,447,851, and 5,112,946; EP 307,434; EP 367,166; PCT publication Nos. WO 96/04388 and WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88: 10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in  
5 their entireties).

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to alter the activities of antibodies of the invention or fragments thereof (*e.g.*, antibodies or fragments thereof with  
10 higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its  
15 entirety). In one embodiment, antibodies or fragments thereof, or the encoded antibodies or fragments thereof, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more portions of a polynucleotide encoding an antibody or antibody fragment, which portions immunospecifically bind to a RSV antigen may be recombined  
20 with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Moreover, the antibodies of the present invention or fragments thereof can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a  
25 pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., 1989, Proc. Natl. Acad. Sci. USA 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin"HA" tag, which corresponds to an epitope derived from  
30 the influenza hemagglutinin protein (Wilson et al., 1984, Cell 37:767) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a RSV infection as part of a clinical testing procedure to, *e.g.*, determine the efficacy of a given treatment regimen.  
35 Detection can be facilitated by coupling the antibody or fragment thereof to a detectable



substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or  
5 fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of  
10 suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of  
15 suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{111}\text{In}$  or  $^{99}\text{Tc}$ .

An antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, *e.g.*, a cytostatic or cytotoxic agent, a therapeutic agent or a radioactive metal ion, *e.g.*, alpha-emitters. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide,  
20 emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine,  
25 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin,  
30 mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

Further, an antibody or fragment thereof may be conjugated to a therapeutic agent or drug moiety that modifies a given biological response. Therapeutic agents or drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the  
35 drug moiety may be a protein or polypeptide possessing a desired biological activity. Such

proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor,  $\alpha$ -interferon,  $\beta$ -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, *e.g.*, TNF- $\alpha$ , TNF- $\beta$ , AIM I (see, International Publication No. WO 97/33899), AIM 5 II (see, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., 1994, J. Immunol., 6:1567-1574), and VEGI (see, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, *e.g.*, angiostatin or endostatin; or, a biological response modifier such as, for example, a lymphokine (*e.g.*, interleukin-1 ("IL- 1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating 10 factor ("GM-CSF"), and granulocyte colony stimulating factor ("G-CSF")), or a growth factor (*e.g.*, growth hormone ("GH")).

Techniques for conjugating such therapeutic moieties to antibodies are well known, see, *e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 15 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of 20 Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., 1982, Immunol. Rev. 62:119-58.

An antibody or fragment thereof, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be 25 used as a therapeutic.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

Antibodies may also be attached to solid supports, which are particularly useful for 30 immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

## 5.2. Prophylactic and Therapeutic Uses of Antibodies

The present invention is directed to antibody-based therapies which involve administering antibodies of the invention or fragments thereof to a mammal, preferably a human, for preventing, treating, or ameliorating one or more symptoms associated with a RSV infection. Prophylactic and therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). Antibodies of the invention or fragments thereof may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

Antibodies of the present invention or fragments thereof that function as antagonists of a RSV infection can be administered to a mammal, preferably a human, to treat, prevent or ameliorate one or more symptoms associated with a RSV infection. For example, antibodies or fragments thereof which disrupt or prevent the interaction between a RSV antigen and its host cell receptor may be administered to a mammal, preferably a human, to treat, prevent or ameliorate one or more symptoms associated with a RSV infection.

In a specific embodiment, an antibody or fragment thereof prevents RSV from binding to its host cell receptor by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to RSV binding to its host cell receptor in the absence of said antibodies or antibody fragments. In another embodiment, a combination of antibodies, a combination of antibody fragments, or a combination of antibodies and antibody fragments prevent RSV from binding to its host cell receptor by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to RSV binding to its host cell receptor in the absence of said antibodies and/or antibody fragments.

Antibodies or fragments thereof which do not prevent RSV from binding its host cell receptor but inhibit or downregulate RSV replication can also be administered to a mammal to treat, prevent or ameliorate one or more symptoms associated with a RSV infection. The ability of an antibody or fragment thereof to inhibit or downregulate RSV replication may be determined by techniques described herein or otherwise known in the art. For example, the inhibition or downregulation of RSV replication can be determined by detecting the RSV titer in the lungs of a mammal, preferably a human.

In a specific embodiment, an antibody of the present invention or fragment thereof inhibits or downregulates RSV replication by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to RSV replication in absence of said antibodies or antibody fragments. In another embodiment, a combination of antibodies, a combination of antibody fragments, or a combination of antibodies and antibody fragments inhibit or downregulate RSV replication by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to RSV replication in absence of said antibodies and/or antibody fragments.

One or more antibodies of the present invention or fragments thereof that immunospecifically bind to one or more RSV antigens may be used locally or systemically in the body as a therapeutic. The antibodies of this invention or fragments thereof may also be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the number or activity of effector cells which interact with the antibodies. The antibodies of this invention or fragments thereof may also be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, *e.g.*, IL-2, IL-3 and IL-7), which, for example, serve to increase the immune response. The antibodies of this invention or fragments thereof may also be advantageously utilized in combination with one or more drugs used to treat RSV infection such as, for example anti-viral agents.

Antibodies of the invention or fragments may be used in combination with one or more of the following drugs: NIH-351 (Gemini Technologies), recombinant RSV vaccine (Aviron), RSVf-2 (Intracel), F-50042 (Pierre Fabre), T-786 (Trimeris), VP-36676 (ViroPharma), RFI-641 (American Home Products), VP-14637 (ViroPharma), PFP-1 and PFP-2 (American Home Products), RSV vaccine (Avant Immunotherapeutics), and F-50077 (Pierre Fabre).

The antibodies of the invention may be administered alone or in combination with other types of treatments (*e.g.*, hormonal therapy, immunotherapy, and anti-inflammatory agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human or humanized antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

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It is preferred to use high affinity and/or potent *in vivo* inhibiting antibodies and/or neutralizing antibodies that immunospecifically bind to a RSV antigen, for both immunoassays directed to RSV, prevention of RSV infection and therapy for RSV infection. It is also preferred to use polynucleotides encoding high affinity and/or potent *in vivo* inhibiting antibodies and/or neutralizing antibodies that immunospecifically bind to a RSV antigen, for both immunoassays directed to RSV and therapy for RSV infection. Such antibodies or fragments thereof will preferably have an affinity for the RSV F glycoprotein and/or fragments of the F glycoprotein.

In one embodiment, therapeutic or pharmaceutical compositions comprising antibodies of the invention or fragments thereof are administered to a mammal, preferably a human, to treat, prevent or ameliorate one or more symptoms associated with RSV infection. In another embodiment, therapeutic or pharmaceutical compositions comprising antibodies of the invention or fragments thereof are administered to a human with cystic fibrosis, bronchopulmonary dysplasia, congenital heart disease, congenital immunodeficiency or acquired immunodeficiency, or to a human who has had a bone marrow transplant to treat, prevent or ameliorate one or more symptoms associated with RSV infection. In another embodiment, therapeutic or pharmaceutical compositions comprising antibodies of the invention or fragments thereof are administered to a human infant, preferably a human infant born prematurely or a human infant at risk of hospitalization for RSV infection to treat, prevent or ameliorate one or more symptoms associated with RSV infection. In yet another embodiment, therapeutic or pharmaceutical compositions comprising antibodies of the invention or fragments thereof are administered to the elderly or people in group homes (*e.g.*, nursing homes or rehabilitation centers).

In a specific embodiment, a mammal, preferably a human, is administered a therapeutic or pharmaceutical composition comprising one or more antibodies of the present invention or fragments thereof for the treatment, prevention or amelioration of one or more symptoms associated with a RSV infection in an amount effective for decreasing RSV titers. In accordance with this embodiment, an effective amount of antibodies or antibody fragments reduces the RSV titers in the lung as measured, for example, by the concentration of RSV in sputum samples or a lavage from the lungs from a mammal. In another embodiment, a mammal, preferably a human, is administered a therapeutic or pharmaceutical composition comprising one or more antibodies of the present invention or fragments thereof for the treatment, prevention or amelioration of symptoms associated with a RSV infection in an amount effective for inducing an immune response in the mammal.

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In another embodiment, a mammal, preferably a human, is administered a first dose of a therapeutic or pharmaceutical composition comprising less than 15 mg/kg, preferably less than 10 mg/kg, less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg or less than 0.5 mg/kg of one or more antibodies or fragments thereof that immunospecifically bind to one or more RSV antigens with higher affinity and/or higher avidity than previously known antibodies (*e.g.*, SYNAGIS®) for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of at least 1 µg/ml, preferably at least 2 µg/ml, at least 5 µg/ml, at least 10 µg/ml, at least 15 µg/ml, at least 20 µg/ml, or at least 25 µg/ml 20 days (preferably 25, 30, 35, 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the serum titer of said antibodies or antibody fragments is less than 30 µg/ml 30 days after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, said antibodies are AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, and A4B4-F52S

In another embodiment, a mammal, preferably a human, is administered a first dose of a therapeutic or pharmaceutical composition comprising less than 15 mg/kg, preferably less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg or less than 0.5 mg/kg of one or more antibodies or fragments thereof which have increased *in vivo* half-lives and which immunospecifically bind to one or more RSV antigens with higher affinity and/or higher avidity than previously known antibodies (*e.g.*, SYNAGIS®) for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of at least 1 µg/ml, preferably at least 2 µg/ml, at least 5 µg/ml, at least 10 µg/ml, at least 15 µg/ml, at least 20 µg/ml, or at least 25 µg/ml 25 days (preferably 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the serum titer of said antibodies or antibody fragments is less than 30 µg/ml 30 days after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the novel antibodies are AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, and A4B4-F52S.

In another embodiment, a mammal, preferably a human, is administered a first dose of a therapeutic or pharmaceutical composition comprising approximately 15 mg/kg of HL-SYNAGIS or antigen-binding fragments thereof for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount

effective to induce a serum titer of at least 1 µg/ml, preferably at least 30 µg/ml, at least 35 µg/ml, at least 40 µg/ml, or at least 50 µg/ml 25 days (preferably 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. In another embodiment, a mammal, preferably a human, is administered a first dose of a  
5 therapeutic or pharmaceutical composition comprising less than 15 mg/kg (preferably 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, or 0.5 mg/kg or less) of HL-SYNAGIS or antigen-binding fragments thereof for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of at least 1 µg/ml, preferably at least 30 µg/ml, at least 35  
10 µg/ml, at least 40 µg/ml, or at least 50 µg/ml 25 days (preferably 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose.

The present invention encompasses therapeutic or pharmaceutical compositions for pulmonary delivery comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with a higher affinity and/or a higher  
15 avidity than previously known antibodies (*e.g.*, SYNAGIS®). The present invention also encompasses therapeutic or pharmaceutical compositions for pulmonary delivery comprising SYNAGIS® or an antigen-binding fragment thereof.

In one embodiment, a mammal, preferably a human, is administered a first dose of a therapeutic or pharmaceutical composition for pulmonary delivery comprising less than 15  
20 mg/kg, preferably less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg or less than 0.5 mg/kg, or less than 0.01 mg/kg of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with higher affinity and/or higher avidity than previously known antibodies (*e.g.*, SYNAGIS®) for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount  
25 effective to induce a titer of 20 ng per mg of lung protein (preferably at least 40 ng/mg, at least 60 ng/mg, at least 80 ng/mg, at least 50 ng/mg, at least 75 ng/mg, at least 100 ng/mg, or at least 150 ng/mg) in an intubation sample or lavage from the lungs of said mammal 20 days (preferably 25, 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the serum titer of said  
30 antibodies or antibody fragments is less than 100 ng/ml of protein 30 days after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the novel antibodies are AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, and A4B4-F52S.

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In another embodiment, a mammal, preferably a human, is administered a first dose of a therapeutic or pharmaceutical composition for pulmonary delivery comprising approximately 15 mg/kg of SYNAGIS® or fragments thereof for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a titer of 20 ng per mg of lung protein (preferably at least 40 ng/mg, at least 60 ng/mg, at least 80 ng/mg, at least 50 ng/mg, at least 75 ng/mg, at least 100 ng/mg, or at least 150 ng/mg) in an intubation sample or lavage from the lungs of said mammal 30 days (preferably 35 or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. In another embodiment, a mammal, preferably a human, is administered a first dose of a therapeutic or pharmaceutical composition for pulmonary delivery comprising less than 15 mg/kg (preferably 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, or 0.5 mg/kg or less) of SYNAGIS® or fragments thereof for the prevention of a RSV infection in an amount effective to induce a titer of 20 ng per mg of lung protein (preferably at least 40 ng/mg, at least 60 ng/mg, at least 80 ng/mg, at least 50 ng/mg, at least 75 ng/mg, at least 100 ng/mg, or at least 150 ng/mg) in an intubation sample or lavage from the lungs of said mammal 30 days (preferably 35 or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose.

The present invention encompasses therapeutic or pharmaceutical compositions for pulmonary delivery comprising one or more antibodies or fragments thereof which have increased *in vivo* half-lives and which immunospecifically bind to one or more RSV antigens with a higher affinity and/or a higher avidity than previously known antibodies (*e.g.*, SYNAGIS®). The present invention also encompasses therapeutic or pharmaceutical compositions for pulmonary delivery comprising HL-SYNAGIS or an antigen-binding fragment thereof.

The present invention encompasses sustained release compositions comprising one or more antibodies or fragments thereof which have increased *in vivo* half-lives and which immunospecifically bind to one or more RSV antigens with a higher affinity and/or a higher avidity than previously known antibodies (*e.g.*, SYNAGIS®). The present invention also encompasses sustained release compositions comprising SYNAGIS® or an antigen-binding fragment thereof.

In one embodiment, a mammal, preferably a human, is administered a first dose of a sustained release formulation comprising less than 15 mg/kg, preferably less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg or less than 0.5 mg/kg of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with higher



affinity and/or higher avidity than previously known antibodies (e.g., SYNAGIS®) for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of at least 1 µg/ml, preferably at least 2 µg/ml, at least 5 µg/ml, at least 10 µg/ml, at least 15 µg/ml, at least 20 µg/ml, or at least 25 µg/ml for at least 10 days (preferably at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the serum titer of said antibodies or antibody fragments is less than 30 µg/ml 30 days after the administration of the first dose and prior to the administration of a subsequent dose. Preferably, the novel antibodies are

5 AFFF, Pf12, P12f4, P11d4, Ale9, A12a6, A13c4, A17d4, A4B4, A8C7, 1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF(1), 6H8, L1-7E5, L2-15B10, A13a11, A1h5, A4B4(1), A4B4L1FR-S28R, or A4B4-F52S.

In another embodiment, a mammal, preferably a human, is administered a first dose of a sustained release formulation comprising less than 15 mg/kg, preferably less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg or less than 0.5 mg/kg of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with higher affinity and/or higher avidity than previously known antibodies (e.g., SYNAGIS®) for the prevention, treatment, or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of 1 µg/ml, preferably 2 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, or 25 µg/ml that is maintained for at least 10 days (preferably at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 days) after the administration of the first dose and prior to the administration of a subsequent dose without exceeding a serum titer of 30 µg/ml.

15 20

In another embodiment, a mammal, preferably a human, is administered a first dose of a sustained release formulation comprising approximately 15 mg/kg of SYNAGIS® or fragments thereof for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a titer of at least 30 µg/ml, preferably at least 35 µg/ml, at least 40 µg/ml, or at least 50 µg/ml 25 days (preferably 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose. In another embodiment, a mammal, preferably a human, is administered a first dose of a sustained release formulation comprising less than 15 mg/kg (preferably 10 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less, or 0.5 mg/kg or less) of SYNAGIS® or fragments thereof for the prevention of a RSV infection in an amount effective to induce at least 30 µg/ml, preferably at least 35 µg/ml, at least 40 µg/ml, or at

25 30

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least 50 µg/ml 25 days (preferably 30, 35, or 40 days) after the administration of the first dose and prior to the administration of a subsequent dose.

In another embodiment, a mammal, preferably a human, is administered a first dose of a sustained release formulation comprising less than 15 mg/kg, preferably less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg or less than 0.5 mg/kg of SYNAGIS® or an antigen-binding fragment thereof for the prevention, treatment, or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of 1 µg/ml, preferably 2 µg/ml, 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, or 25 µg/ml that is maintained for at least 10 days (preferably at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 days) after the administration of the first dose and prior to the administration of a subsequent dose without exceeding a serum titer of 30 µg/ml.

The present invention encompasses sustained release formulations comprising one or more antibodies or fragments thereof which have increased *in vivo* half-lives and which immunospecifically bind to one or more RSV antigens with a higher affinity and/or a higher avidity than previously known antibodies such as, *e.g.*, SYNAGIS®. The present invention also encompasses sustained release formulations comprising HL-SYNAGIS or an antigen-binding fragment thereof.

The present invention encompasses sustained release formulations for pulmonary delivery comprising one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens with a higher affinity and/or a higher avidity than previously known antibodies (*e.g.*, SYNAGIS®). The present invention also encompasses sustained release formulations for pulmonary delivery comprising one or more antibodies or fragments thereof which have increased *in vivo* half-lives and which immunospecifically bind to one or more RSV antigens with a higher affinity and/or a higher avidity than previously known antibodies (*e.g.*, SYNAGIS®). The present invention also encompasses sustained release formulations for pulmonary delivery comprising SYNAGIS® or fragments thereof. The present invention further encompasses sustained release formulations for pulmonary delivery comprising HL-SYNAGIS or an antigen-binding fragment thereof.

In another embodiment, a mammal, preferably a human, is administered a first dose of a therapeutic or pharmaceutical composition comprising less than 10 mg/kg, less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg or less than 0.5 mg/kg of one or more antibodies of the present invention or fragments thereof for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of at least 35 µg/ml, at least 40 µg/ml, at least 50 µg/ml, at least 80 µg/ml, at least 100 µg/ml, at least 120 µg/ml, at least 150 µg/ml, at least 200 µg/ml,

at least 250 µg/ml, or at least 300 µg/ml 20 days (preferably 25, 30, 35 or 40 days) after the administration of the first dose. In another embodiment, a mammal, preferably a human, is administered a first dose of a therapeutic or pharmaceutical composition comprising approximately 15 mg/kg of one or more antibodies of the present invention or fragments thereof for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a serum titer of at least 100 µg/ml, at least 125 µg/ml, at least 150 µg/ml, at least 200 µg/ml, at least 250 µg/ml, at least 300 µg/ml, at least 350 µg/ml, at least 400 µg/ml, or at least 450 µg/ml 20 days (preferably 25, 30, 35 or 40 days) after the administration of the first dose. The term "approximately 15 mg/kg" as used herein refers to a range of between 14 mg/kg and 16 mg/kg.

In another embodiment, a mammal, preferably a human, is administered a dose of a pharmaceutical composition comprising one or more antibodies of the present invention or fragments thereof for the prevention a RSV infection in an amount effective to induce a prophylactically effective serum titer of less than 10 µg/ml, less than 8 µg/ml, less than 5 µg/ml, less than 3 µg/ml, less than 1 µg/ml, or less than 0.5 µg/ml 30 days after the administration of the dose, wherein said prophylactically effective serum titer is the serum titer that reduces the incidence of RSV infection in the human or the serum titer in a cotton rat that results in a RSV titer 5 days after challenge with 10<sup>5</sup> pfu RSV that is 99% lower than the RSV titer in the cotton rat 5 days after challenge with 10<sup>5</sup> pfu of RSV in a cotton rat not administered the dose prior to challenge. Preferably, the dose of the pharmaceutical composition comprises less than 10 mg/kg, less than 5 mg/kg, less than 3 mg/kg, less than 1 mg/kg, or less than 0.5 mg/kg of one or more antibodies of the present invention or fragments thereof.

In yet another embodiment, a mammal, preferably a human, is administered a dose of a therapeutic or pharmaceutical composition comprising one or more antibodies of the present invention or fragments thereof for the treatment or amelioration of one or more symptoms associated with a RSV infection in an amount effective to induce a therapeutically effective serum titer of less than 10 µg/ml, less than 8 µg/ml, less than 5 µg/ml, less than 3 µg/ml, less than 1 µg/ml, or less than 0.5 µg/ml 30 days after the administration of the dose, wherein said therapeutically effective serum titer is the serum titer that reduces the severity or length of RSV infection or is the serum titer in a cotton rat that results in a RSV titer in the rat 5 days after challenge with 10<sup>5</sup> pfu RSV that is 99% lower than the RSV titer 5 days after challenge with 10<sup>5</sup> pfu of RSV in a cotton rat not administered the dose prior to challenge. Preferably, the dose of the therapeutic or pharmaceutical composition comprises less than 12 mg/kg, less than 10 mg/kg, less than 5

mg/kg, less than 3 mg/kg, less than 1 mg/kg, or less than 0.5 mg/kg of one or more antibodies of the present invention or fragments thereof.

### **5.3. Methods of Administration of Antibodies**

5       The invention provides methods of treatment, prophylaxis, and amelioration of one or more symptoms associated with RSV infection by administering to a subject of an effective amount of antibody or fragment thereof, or pharmaceutical composition comprising an antibody of the invention or fragment thereof. In a preferred aspect, an antibody or fragment thereof is substantially purified (*i.e.*, substantially free from substances  
10 that limit its effect or produce undesired side-effects). The subject is preferably a mammal such as non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats etc.) and a primate (*e.g.*, monkey such as a cynomolgous monkey and a human). In a preferred embodiment, the subject is a human. In another preferred embodiment, the subject is a human infant or a human infant born prematurely. In another embodiment, the subject is a human with cystic  
15 fibrosis, bronchopulmonary dysplasia, congenital heart disease, congenital immunodeficiency or acquired immunodeficiency, a human who has had a bone marrow transplant, or an elderly human

Various delivery systems are known and can be used to administer an antibody of the invention or a fragment thereof, *e.g.*, encapsulation in liposomes, microparticles,  
20 microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering an antibody or fragment thereof, or pharmaceutical composition include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular,  
25 intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, antibodies of the present invention or fragments thereof, or pharmaceutical compositions are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous  
30 linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968, 5,985, 320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and  
35 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO

98/31346, and WO 99/66903, each of which is incorporated herein by reference their entirety. In a preferred embodiment, an antibody of the invention or fragment thereof, or composition of the invention is administered using Alkermes AIR™ pulmonary drug delivery technology (Alkermes, Inc., Cambridge, MA).

5           The invention provides for any method of administering lower doses of known antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens than previously thought to be effective for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection. Preferably, lower doses of known antibodies or fragments thereof which immunospecifically bind to one or  
10 more RSV antigens are administered by pulmonary administration. The present invention also provides for any method of administering a novel antibody of the invention or fragment thereof for the prevention, treatment or amelioration of one or more symptoms associated with a RSV infection. Preferably, novel antibodies of the invention or fragments thereof are administered by pulmonary administration.

15           The invention also provides that an antibody or fragment thereof is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of antibody or antibody fragment. In one embodiment, the antibody or antibody fragment is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate  
20 concentration for administration to a subject. Preferably, the antibody or antibody fragment is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, or at least 75 mg. The lyophilized antibody or antibody fragment should be stored at between 2 and 8°C in its original container and the  
25 antibody or antibody fragment should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, an antibody or fragment thereof is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the antibody or antibody fragment. Preferably, the liquid form of the antibody or fragment thereof is  
30 supplied in a hermetically sealed container at least 1 mg/ml, more preferably at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, or at least 25 mg/ml.

          In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved  
35 by, for example, and not by way of limitation, local infusion, by injection, or by means of an

implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering an antibody of the invention or fragment thereof, care must be taken to use materials to which the antibody or antibody fragment does not absorb.

5 In another embodiment, the composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 3 17-327; see generally *ibid.*).

In yet another embodiment, the composition can be delivered in a controlled release  
10 or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:20; Buchwald *et al.*, 1980, *Surgery* 88:507; Saudek *et al.*, 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the antibodies of the invention or fragments thereof (see *e.g.*, *Medical*  
15 *Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, *Ann. Neurol.* 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 7 1:105); U.S. Patent No.  
20 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate),  
25 poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release  
30 system can be placed in proximity of the therapeutic target, *i.e.*, the lungs, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

Controlled release systems are discussed in the review by Langer (1990, *Science* 249:1527-1533). Any technique known to one of skill in the art can be used to produce  
35 sustained release formulations comprising one or more antibodies of the invention or

fragments thereof. See, *e.g.*, U.S. Patent No. 4,526,938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning *et al.*, 1996, "Intratumoral Radioimmunotherapy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," *Radiotherapy & Oncology* 39:179-189, Song *et al.*, 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," *PDA Journal of Pharmaceutical Science & Technology* 50:372-397, Cleek *et al.*, 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854, and Lam *et al.*, 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760, each of which is incorporated herein by reference in their entirety.

In a specific embodiment where the composition of the invention is a nucleic acid encoding an antibody or antibody fragment, the nucleic acid can be administered *in vivo* to promote expression of its encoded antibody or antibody fragment, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see *e.g.*, Joliot *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a prophylactically or therapeutically effective amount of an antibody or a fragment thereof, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete)), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica

gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, 5 powders, sustained-release formulations and the like. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a prophylactically or therapeutically effective 10 amount of the antibody or fragment thereof, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to 15 human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection.

Generally, the ingredients of compositions of the invention are supplied either 20 separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile 25 water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with 30 cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the composition of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a RSV infection can be determined by standard clinical techniques. For example, the dosage of the 35 composition which will be effective in the treatment, prevention or amelioration of one or



more symptoms associated with a RSV infection can be determined by administering the composition to a cotton rat, measuring the RSV titer after challenging the cotton rat with  $10^5$  pfu of RSV and comparing the RSV titer to that obtain for a cotton rat not administered the composition. Accordingly, a dosage that results in a 2 log decrease or a 99% reduction in RSV titer in the cotton rat challenged with  $10^5$  pfu of RSV relative to the cotton rat challenged with  $10^5$  pfu of RSV but not administered the composition is the dosage of the composition that can be administered to a human for the treatment, prevention or amelioration of symptoms associated with RSV infection. The dosage of the composition which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a RSV infection can be determined by administering the composition to an animal model (*e.g.*, a cotton rat or monkey) and measuring the serum titer of antibodies or fragments thereof that immunospecifically bind to a RSV antigen. Accordingly, a dosage of the composition that results in a serum titer of at least 1  $\mu\text{g/ml}$ , preferably 2  $\mu\text{g/ml}$ , 5  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$ , 20  $\mu\text{g/ml}$ , 25  $\mu\text{g/ml}$ , at least 35  $\mu\text{g/ml}$ , at least 40  $\mu\text{g/ml}$ , at least 50  $\mu\text{g/ml}$ , at least 75  $\mu\text{g/ml}$ , at least 100  $\mu\text{g/ml}$ , at least 125  $\mu\text{g/ml}$ , at least 150  $\mu\text{g/ml}$ , at least 200  $\mu\text{g/ml}$ , at least 250  $\mu\text{g/ml}$ , at least 300  $\mu\text{g/ml}$ , at least 350  $\mu\text{g/ml}$ , at least 400  $\mu\text{g/ml}$ , or at least 450  $\mu\text{g/ml}$  can be administered to a human for the treatment, prevention or amelioration of one or more symptoms associated with RSV infection. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges.

The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the RSV infection, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model (*e.g.*, the cotton rat or Cynomolgous monkey) test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention or fragments thereof may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the lung) of the antibodies by modifications such as, for example, lipidation.

In a specific embodiment, antibodies of the invention or fragments thereof, or compositions comprising antibodies of the invention or fragments thereof are administered once a month just prior to or during the RSV season. In another embodiment, antibodies of the invention or fragments thereof, or compositions comprising antibodies of the invention  
5 or fragments thereof are administered every two months just prior to or during the RSV season. In yet another embodiment, antibodies of the invention or fragments thereof, or compositions comprising antibodies of the invention or fragments thereof are administered once just prior to or during the RSV season. The term "RSV season" refers to the season when RSV infection is most likely to occur. Typically, the RSV season in the northern  
10 hemisphere commences in November and lasts through April.

In one embodiment, approximately 5 mg/kg or less (preferably 1.5 mg/kg or less) of an antibody or fragment thereof which immunospecifically binds to a RSV antigen with a higher avidity and/or higher affinity than previously known antibodies such as, *e.g.*, SYNAGIS®, is administered five times, 3 times, or 1 to 2 times during a RSV season to a  
15 mammal, preferably a human. In another embodiment, approximately 1.5 mg/kg of an antibody or a fragment thereof which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity than known antibodies such as, *e.g.*, SYNAGIS®, is administered monthly five times during an RSV season to a mammal, preferably a human, intramuscularly. In another embodiment, 3 mg/kg of an antibody or a fragment thereof  
20 which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity than known antibodies such as, *e.g.*, SYNAGIS® is administered monthly three times during an RSV season to a mammal, preferably a human, intramuscularly. In yet another embodiment, 5 mg/kg of an antibody or a fragment thereof which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity  
25 than known antibodies such as, *e.g.*, SYNAGIS® is administered monthly one to two times during an RSV season to a mammal, preferably a human, intramuscularly.

In a specific embodiment, 15 mg/kg of HL-SYNAGIS or an antigen-binding fragment thereof is administered to a mammal, preferably a human, intramuscularly five times during an RSV season. In another embodiment, approximately 5 mg/kg or less  
30 (preferably 1.5 mg/kg or less) of an antibody or fragment thereof which immunospecifically binds to a RSV antigen with a higher avidity and/or higher affinity than previously known antibodies such as, *e.g.*, SYNAGIS®, is administered five times, 3 times, or 1 to 2 times during a RSV season to a mammal, preferably a human. In another embodiment, 3 mg/kg of antibody or a fragment thereof which immunospecifically binds to a RSV antigen with a  
35 higher avidity and/or a higher affinity known antibodies such as, *e.g.*, SYNAGIS® and

which has an increased *in vivo* half-life is administered monthly three times during an RSV season to a mammal, preferably a human, intramuscularly. In another embodiment, 5 mg/kg of antibody or a fragment thereof which immunospecifically binds to a RSV antigen with a higher avidity and/or a higher affinity than known antibodies such as, *e.g.*, SYNAGIS® and  
5 which has an increased *in vivo* half-life is administered to a mammal, preferably a human, intramuscularly twice times during an RSV season.

In a specific embodiment, an approximately 15 mg/kg bolus of SYNAGIS® or an antigen-binding fragment thereof not in a sustained release formulation is administered to a mammal, preferably a human, and after a certain period of time less than 15 mg/kg  
10 (preferably 5 mg/kg or less, more preferably 3 mg/kg or less, and most preferably 1.5 mg/kg or less) of SYNAGIS® or an antibody fragment in a sustained release is administered to said mammal intramuscularly two, three or four times during an RSV season. In accordance with this embodiment, a certain period of time can be 1 to 5 days, a week, two weeks, or a month. In another embodiment, approximately 15 mg/kg or less (preferably at least 2  
15 mg/kg, at least 5 mg/kg, or at least 10 mg/kg) of SYNAGIS® or an antigen-binding fragment thereof in a sustained release formulation is administered to a mammal, preferably a human, intramuscularly two, three or four times during an RSV season.

In another embodiment, approximately 15 mg/kg or less (preferably at least 2 mg/kg, at least 5 mg/kg, or at least 10 mg/kg) of one or more antibodies or fragments thereof which  
20 immunospecifically bind to one or more RSV antigens is administered to the lungs of a mammal by pulmonary delivery and then after a certain period of time (*e.g.*, 15 minutes, 30 minutes, 45 minutes, 1 hour, 6 hours, 12 hours, 1 day, 5 days, 10 days, 20 days, 25 days, 30 days, or 40 days) approximately 15 mg/kg or less of one or more said antibodies or antibody fragments is administered intramuscularly said mammal. In another embodiment,  
25 approximately 15 mg/kg or less (preferably at least 2 mg/kg, at least 5 mg/kg, or at least 10 mg/kg) of one or more antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens is administered to a mammal intramuscularly and then after a certain period of time (*e.g.*, 15 minutes, 30 minutes, 45 minutes, 1 hour, 6 hours, 12 hours, 1 day, 5 days, 10 days, 20 days, 25 days, 30 days, or 40 days) approximately 15 mg/kg or less  
30 of one or more said antibodies or antibody fragments is administered to the lungs of said mammal.

### 5.3.1. Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies  
35 or functional derivatives thereof, are administered to treat, prevent or ameliorate one or

more symptoms associated with RSV infection, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded antibody or antibody fragment that mediates a prophylactic or therapeutic effect.

5 Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, Science 260:926-932 (1993); and  
10 Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

15 In a preferred aspect, a composition of the invention comprises nucleic acids encoding an antibody, said nucleic acids being part of an expression vector that expresses the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, operably linked to the antibody coding region, said promoter being inducible or constitutive,  
20 and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989,  
25 Nature 342:435-438). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a subject may be either direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in  
30 which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the subject. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by  
35 any of numerous methods known in the art, *e.g.*, by constructing them as part of an

appropriate nucleic acid expression vector and administering it so that they become intracellular, *e.g.*, by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180; WO 92/22635; W092/203 16; W093/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; and Zijlstra et al., 1989, Nature 342:435-438).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention or fragments thereof are used. For example, a retroviral vector can be used (see Miller et al., 1993, Meth. Enzymol. 217:581-599). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a subject. More detail about retroviral vectors can be found in Boesen et al., 1994, Biotherapy 6:291-302, which describes the use of a retroviral vector to deliver the *mdr 1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, J. Clin. Invest. 93:644-651; Klein et al., 1994, Blood 83:1467-1473; Salmons and Gunzberg, 1993, Human Gene Therapy 4:129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3:110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing

cells. Kozarsky and Wilson, 1993, Current Opinion in Genetics and Development 3:499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, Human Gene Therapy 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, Science 252:431-434; Rosenfeld et al., 1992, Cell 68:143-155; Mastrangeli et al., 1993, J. Clin. Invest. 91:225-234; PCT Publication W094/12649; and Wang et al., 1995, Gene Therapy 2:775-783. In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, Proc. Soc. Exp. Biol. Med. 204:289-300; and U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, 1993, Meth. Enzymol. 217:599-618; Cohen et al., 1993, Meth. Enzymol. 217:618-644; Clin. Pharma. Ther. 29:69-92 (1985)) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a subject by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the subject.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody or fragment thereof are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention (see *e.g.*, PCT Publication WO 94/08598; Stemple and Anderson, 1992, Cell 71:973-985; Rheinwald, 1980, Meth. Cell Bio. 21A:229; and Pittelkow and Scott, 1986, Mayo Clinic Proc. 61:771).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

#### **5.4. Antibody Characterization and Demonstration of Therapeutic or Prophylactic Utility**

Antibodies of the present invention or fragments thereof may be characterized in a variety of ways. In particular, antibodies of the invention or fragments thereof may be assayed for the ability to immunospecifically bind to a RSV antigen. Such an assay may be performed in solution (*e.g.*, Houghten, 1992, Bio/Techniques 13:412-421), on beads (Lam, 1991, Nature 354:82-84), on chips (Fodor, 1993, Nature 364:555-556), on bacteria (U.S. Patent No. 5,223,409), on spores (U.S. Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (Cull et al., 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or on phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310) (each of these references is incorporated herein in its entirety by reference). Antibodies or fragments thereof that have been identified to immunospecifically bind to a

RSV antigen or a fragment thereof can then be assayed for their specificity and affinity for a RSV antigen.

The antibodies of the invention or fragments thereof may be assayed for immunospecific binding to a RSV antigen and cross-reactivity with other antigens by any method known in the art. Immunoassays which can be used to analyze immunospecific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 40° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), blocking the membrane with primary antibody (the



antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, *e.g.*, an anti-human antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in  
5 blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

10 ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a  
15 second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be  
20 modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a  
25 competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of the present invention or a fragment thereof for a RSV antigen and the binding off-rates can be determined from the data by scatchard plot analysis.

30 Competition with a second antibody can also be determined using radioimmunoassays. In this case, a RSV antigen is incubated with an antibody of the present invention or a fragment thereof conjugated to a labeled compound (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second antibody.

In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding  
35 on and off rates of antibodies or fragments thereof to a RSV antigen. BIAcore kinetic

analysis comprises analyzing the binding and dissociation of a RSV antigen from chips with immobilized antibodies or fragments thereof on their surface (see the Example section *infra*).

The antibodies of the invention or fragments thereof can also be assayed for their ability to inhibit the binding of RSV to its host cell receptor using techniques known to those of skill in the art. For example, cells expressing the receptor for RSV can be contacted with RSV in the presence or absence of an antibody or fragment thereof and the ability of the antibody or fragment thereof to inhibit RSV's binding can be measured by, for example, flow cytometry or a scintillation assay. RSV (*e.g.*, a RSV antigen such as F glycoprotein or G glycoprotein) or the antibody or antibody fragment can be labeled with a detectable compound such as a radioactive label (*e.g.*,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , and  $^{125}\text{I}$ ) or a fluorescent label (*e.g.*, fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine) to enable detection of an interaction between RSV and its host cell receptor. Alternatively, the ability of antibodies or fragments thereof to inhibit RSV from binding to its receptor can be determined in cell-free assays. For example, RSV or a RSV antigen such as G glycoprotein can be contacted with an antibody or fragment thereof and the ability of the antibody or antibody fragment to inhibit RSV or the RSV antigen from binding to its host cell receptor can be determined. Preferably, the antibody or the antibody fragment is immobilized on a solid support and RSV or a RSV antigen is labeled with a detectable compound. Alternatively, RSV or a RSV antigen is immobilized on a solid support and the antibody or fragment thereof is labeled with a detectable compound. RSV or a RSV antigen may be partially or completely purified (*e.g.*, partially or completely free of other polypeptides) or part of a cell lysate. Further, an RSV antigen may be a fusion protein comprising the RSV antigen and a domain such as glutathione-S-transferase. Alternatively, an RSV antigen can be biotinylated using techniques well known to those of skill in the art (*e.g.*, biotinylation kit, Pierce Chemicals; Rockford, IL).

The antibodies of the invention or fragments thereof can also be assayed for their ability to inhibit or downregulate RSV replication using techniques known to those of skill in the art. For example, RSV replication can be assayed by a plaque assay such as described, *e.g.*, by Johnson et al., 1997, Journal of Infectious Diseases 176:1215-1224. The antibodies of the invention or fragments thereof can also be assayed for their ability to inhibit or downregulate the expression of RSV polypeptides. Techniques known to those of skill in the art, including, but not limited to, Western blot analysis, Northern blot analysis, and RT-PCR can be used to measure the expression of RSV polypeptides. Further, the

antibodies of the invention or fragments thereof can be assayed for their ability to prevent the formation of syncytia.

The antibodies of the invention or fragments thereof are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans.

- 5 For example, *in vitro* assays which can be used to determine whether administration of a specific antibody or composition of the present invention is indicated, include *in vitro* cell culture assays in which a subject tissue sample is grown in culture, and exposed to or otherwise administered an antibody or composition of the present invention, and the effect of such an antibody or composition of the present invention upon the tissue sample is
- 10 observed. In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a RSV infection (e.g., respiratory epithelial cells), to determine if an antibody or composition of the present invention has a desired effect upon such cell types. Preferably, the antibodies or compositions of the invention are also tested in *in vitro* assays and animal model systems prior to administration to humans.
- 15 In a specific embodiment, cotton rats are administered an antibody the invention or fragment thereof, or a composition of the invention, challenged with  $10^5$  pfu of RSV, and four or more days later the rats are sacrificed and RSV titer and anti-RSV antibody serum titer is determined. Further, in accordance with this embodiment, the tissues (e.g., the lung tissues) from the sacrificed rats can be examined for histological changes.

- 20 In accordance with the invention, clinical trials with human subjects need not be performed in order to demonstrate the prophylactic and/or therapeutic efficacy of antibodies of the invention or fragments thereof. *In vitro* and animal model studies using the antibodies or fragments thereof can be extrapolated to humans and are sufficient for demonstrating the prophylactic and/or therapeutic utility of said antibodies or antibody
- 25 fragments.

Antibodies or compositions of the present invention for use in therapy can be tested for their toxicity in suitable animal model systems, including but not limited to rats, mice, cows, monkeys, and rabbits. For *in vivo* testing of an antibody or composition's toxicity any animal model system known in the art may be used.

- 30 Efficacy in treating or preventing viral infection may be demonstrated by detecting the ability of an antibody or composition of the invention to inhibit the replication of the virus, to inhibit transmission or prevent the virus from establishing itself in its host, to reduce the incidence of RSV infection, or to prevent, ameliorate or alleviate one or more symptoms associated with RSV infection. The treatment is considered therapeutic if there
- 35 is, for example, a reduction in viral load, amelioration of one or more symptoms, a reduction

in the duration of a RSV infection, or a decrease in mortality and/or morbidity following administration of an antibody or composition of the invention. Further, the treatment is considered therapeutic if there is an increase in the immune response following the administration of one or more antibodies or fragments thereof which immunospecifically  
5 bind to one or more RSV antigens.

Antibodies or compositions of the invention can be tested *in vitro* and *in vivo* for the ability to induce the expression of cytokines such as IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 and IL-15. Techniques known to those of skill in the art can be used to measure the level of expression of cytokines. For example, the level  
10 of expression of cytokines can be measured by analyzing the level of RNA of cytokines by, for example, RT-PCR and Northern blot analysis, and by analyzing the level of cytokines by, for example, immunoprecipitation followed by western blot analysis and ELISA. In a preferred embodiment, an antibody or composition of the invention is tested for its ability to induce the expression of IFN- $\gamma$ .

15 Antibodies or compositions of the invention can be tested *in vitro* and *in vivo* for their ability to modulate the biological activity of immune cells, preferably human immune cells (*e.g.*, T-cells, B-cells, and Natural Killer cells). The ability of an antibody or composition of the invention to modulate the biological activity of immune cells can be assessed by detecting the expression of antigens, detecting the proliferation of immune  
20 cells, detecting the activation of signaling molecules, detecting the effector function of immune cells, or detecting the differentiation of immune cells. Techniques known to those of skill in the art can be used for measuring these activities. For example, cellular proliferation can be assayed by  $^3\text{H}$ -thymidine incorporation assays and trypan blue cell counts. Antigen expression can be assayed, for example, by immunoassays including, but  
25 are not limited to, competitive and non-competitive assay systems using techniques such as western blots, immunohistochemistry radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays,  
30 protein A immunoassays and FACS analysis. The activation of signaling molecules can be assayed, for example, by kinase assays and electrophoretic shift assays (EMSAs).

Antibodies or compositions of the invention can also be tested for their ability to inhibit viral replication or reduce viral load in *in vitro*, *ex vivo* and *in vivo* assays.

Antibodies or compositions of the invention can also be tested for their ability to decrease  
35 the time course of RSV infection. Antibodies or compositions of the invention can also be

tested for their ability to increase the survival period of humans suffering from RSV infection by at least 25%, preferably at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99%. Further, antibodies or compositions of the invention can be tested for their ability reduce the hospitalization period of humans suffering from RSV infection by at least 60%, preferably at least 75%, at least 85%, at least 95%, or at least 99%. Techniques known to those of skill in the art can be used to analyze the function of the antibodies or compositions of the invention *in vivo*.

### 5.5. Diagnostic Uses of Antibodies

Labeled antibodies, fragments and derivatives and analogs thereof, which immunospecifically bind to a RSV antigen can be used for diagnostic purposes to detect, diagnose, or monitor a RSV infection. The invention provides for the detection of a RSV infection, comprising: (a) assaying the expression of a RSV antigen in cells or a tissue sample of a subject using one or more antibodies or fragments thereof that immunospecifically bind to the RSV antigen; and (b) comparing the level of the RSV antigen with a control level, *e.g.*, levels in normal tissue samples not infected with RSV, whereby an increase in the assayed level of RSV antigen compared to the control level of the RSV antigen is indicative of a RSV infection.

The invention provides a diagnostic assay for diagnosing a RSV infection, comprising: (a) assaying for the level of a RSV antigen in cells or a tissue sample of an individual using one or more antibodies or fragments thereof that immunospecifically bind to a RSV antigen; and (b) comparing the level of the RSV antigen with a control level, *e.g.*, levels in normal tissue samples not infected with RSV, whereby an increase in the assayed RSV antigen level compared to the control level of the RSV antigen is indicative of a RSV infection. A more definitive diagnosis of RSV infection may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of RSV infection.

Antibodies of the invention or fragments thereof can be used to assay RSV antigen levels in a biological sample using classical immunohistological methods as described herein or as known to those of skill in the art (*e.g.*, see Jalkanen et al., 1985, J. Cell. Biol. 101:976-985; and Jalkanen et al., 1987, J. Cell. Biol. 105:3087-3096). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium

(<sup>3</sup>H), indium (<sup>121</sup>In), and technetium (<sup>99</sup>Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a RSV infection in a human. In one embodiment, diagnosis comprises: a) administering (for example,  
5 parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled antibody or fragment thereof that immunospecifically binds to a RSV antigen; b) waiting for a time interval following the administering for permitting the labeled antibody or fragment thereof to preferentially concentrate at sites in the subject (*e.g.*, the lungs) where the RSV antigen is expressed (and for unbound labeled molecule to be cleared to  
10 background level); c) determining background level; and d) detecting the labeled antibody or fragment thereof in the subject, such that detection of labeled antibody or fragment thereof above the background level indicates that the subject has a RSV infection. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular  
15 system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99</sup>Tc. The labeled antibody or  
20 antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

25 Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or  
30 5 to 10 days.

In one embodiment, monitoring of a RSV infection is carried out by repeating the method for diagnosing the RSV infection, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the subject using methods known  
35 in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled

artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

- 5 In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal  
10 and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

#### **5.6. Methods Producing Antibodies**

- 15 The antibodies of the invention or fragments thereof can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Polyclonal antibodies to a RSV antigen can be produced by various procedures well known in the art. For example, a RSV antigen can be administered to various host animals  
20 including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the RSV antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions,  
25 peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a  
30 combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The  
35 term "monoclonal antibody" as used herein is not limited to antibodies produced through

hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma  
5 technology are routine and well known in the art. Briefly, mice can be immunized with a RSV antigen and once an immune response is detected, *e.g.*, antibodies specific for the RSV antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are  
10 selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal  
15 antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with a RSV antigen with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a RSV antigen.

20 Antibody fragments which recognize specific RSV epitopes may be generated by any technique known to those of skill in the art. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region  
25 and the CH1 domain of the heavy chain. Further, the antibodies of the present invention can also be generated using various phage display methods known in the art.

In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries  
30 (*e.g.*, human or murine cDNA libraries of lymphoid tissues). The DNA encoding the VH and VL domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector (*e.g.*, p CANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually  
35 recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen



binding domain that binds to a RSV antigen of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., 1995, J. Immunol. Methods 182:41-50; Ames et al., 1995, J. Immunol. Methods 184:177-186; Kettleborough et al., 1994, Eur. J. Immunol. 24:952-958; Persic et al., 1997, Gene 187:9-18; Burton et al., 1994, Advances in Immunology 57:191-280; PCT application No. PCT/GB91/O1 134; PCT publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described below. Techniques to recombinantly produce Fab, Fab' and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax et al., 1992, BioTechniques 12(6):864-869; Sawai et al., 1995, AJRI 34:26-34; and Better et al., 1988, Science 240:1041-1043 (said references incorporated by reference in their entireties).

To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors expressing a VH constant region, *e.g.*, the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.*, human kappa or lambda constant regions. Preferably, the vectors for expressing the VH or VL domains comprise an EF-1 $\alpha$  promoter, a secretion signal, a cloning site for the variable domain, constant domains, and a selection marker such as neomycin. The VH and VL domains may also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

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For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use human or chimeric antibodies. Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Human antibodies can be made by a variety of methods known in the art including phage display  
5 methods described above using antibody libraries derived from human immunoglobulin sequences. See also U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable  
10 of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the  
15 human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J<sub>H</sub> region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric  
20 mice. The chimeric mice are then be bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes  
25 harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human  
30 antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, PCT publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be

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engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a variable  
5 region derived from a human antibody and a non-human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, 1985, Science 229:1202; Oi et al., 1986, BioTechniques 4:214; Gillies et al., 1989, J. Immunol. Methods 125:191-202; and U.S. Patent Nos. 5,807,715, 4,816,567, and 4,816,397, which are incorporated herein by reference in their entirety. Chimeric antibodies comprising one  
10 or more CDRs from human species and framework regions from a non-human immunoglobulin molecule can be produced using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, Molecular Immunology 28(4/5):489-498; Studnicka et  
15 al., 1994, Protein Engineering 7(6):805-814; and Roguska et al., 1994, PNAS 91:969-973), and chain shuffling (U.S. Patent No. 5,565,332). In a preferred embodiment, chimeric antibodies comprise a human CDR3 having an amino acid sequence of any one of the CDR3 listed in Table 2 and non-human framework regions. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR  
20 donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, Queen et al., U.S. Patent No. 5,585,089; and Riechmann et  
25 al., 1988, Nature 332:323, which are incorporated herein by reference in their entireties.)

Further, the antibodies of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" RSV antigens using techniques well known to those skilled in the art. (See, *e.g.*, Greenspan & Bona, 1989, FASEB J. 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example, antibodies of the invention  
30 which bind to and competitively inhibit the binding of RSV (as determined by assays well known in the art and disclosed in *supra*) to its host cell receptor can be used to generate anti-idiotypes that "mimic" a RSV antigen binding domain and, as a consequence, bind to and neutralize RSV and/or its host cell receptor. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize RSV. For

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example, such anti-idiotypic antibodies can be used to bind RSV and/or to bind its host cell receptors, and thereby block infection.

#### 5.6.1. Polynucleotides Encoding an Antibody

5       The invention provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention or a fragment thereof. The invention also encompasses polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions, *e.g.*, as defined *supra*, to polynucleotides that encode an antibody of the invention.

10       The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. Since the amino acid sequences of the antibodies are known (as described in Table 2), nucleotide sequences encoding these antibodies can be determined using methods well known in the art, *i.e.*, nucleotide codons known to encode particular amino acids are assembled in such a way to  
15       generate a nucleic acid that encodes the antibody or fragment thereof of the invention. Such a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then  
20       amplification of the ligated oligonucleotides by PCR.

      Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a  
25       suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A<sup>+</sup> RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to  
30       identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

      Once the nucleotide sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the  
35       manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed

mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their  
5 entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, one or more of the CDRs is inserted within framework regions using routine recombinant DNA techniques. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework  
10 regions (see, *e.g.*, Chothia et al., 1998, J. Mol. Biol. 278: 457-479 for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to a RSV antigen. Preferably, as discussed *supra*, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the  
15 antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

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#### 5.6.2. Recombinant Expression of an Antibody

Recombinant expression of an antibody of the invention, derivative or analog thereof, (*e.g.*, a heavy or light chain of an antibody of the invention or a portion thereof or a single chain antibody of the invention), requires construction of an expression vector  
25 containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably, but not necessarily, containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods  
30 for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic  
35 recombination. The invention, thus, provides replicable vectors comprising a nucleotide

sequence encoding an antibody molecule of the invention, a heavy or light chain of an antibody, a heavy or light chain variable domain of an antibody or a portion thereof, or a heavy or light chain CDR, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT  
5 Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the  
10 invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention or fragments thereof, or a heavy or light chain thereof, or portion thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for  
15 expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention (see, e.g., U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected  
20 with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing  
25 antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS,  
30 CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are  
35 used for the expression of a recombinant antibody molecule. For example, mammalian

cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 1986, Gene 45:101; and Cockett et al., 1990, Bio/Technology 8:2). In a specific embodiment, the expression of nucleotide  
5 sequences encoding antibodies or fragments thereof which immunospecifically bind to one or more RSV antigens is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example,  
10 when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO 12:1791), in which the antibody coding sequence may be ligated individually  
15 into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by  
20 adsorption and binding to matrix glutathione agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is  
25 used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be  
30 utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a  
35 recombinant virus that is viable and capable of expressing the antibody molecule in infected

hosts (*e.g.*, see Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:355-359). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences.

Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bittner et al., 1987, Methods in Enzymol. 153:51-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, Hela, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT20 and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL7030 and HsS78Bst cells.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the antibody molecule.



A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., 1977, Cell 11:223), hypoxanthineguanine phosphoribosyltransferase (Szybalska & Szybalski, 1992, Proc. Natl. Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, Cell 22:8-17) genes can be employed in tk-, hgp<sup>r</sup>t- or ap<sup>r</sup>t- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler et al., 1980, Natl. Acad. Sci. USA 77:357; O'Hare et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62: 191-217; May, 1993, TIB TECH 11(5):155-2 15); and *hygro*, which confers resistance to hygromycin (Santerre et al., 1984, Gene 30:147). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel *et al.* (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., 1981, J. Mol. Biol. 150:1, which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy

chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; and Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2 197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by recombinant  
5 expression, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the antibodies of the present invention or fragments  
10 thereof may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

### 5.7. Kits

The invention also provides a pharmaceutical pack or kit comprising one or more  
15 containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

20 The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In an alternative embodiment, a kit comprises an antibody fragment that immunospecifically binds to a RSV antigen. In a specific embodiment, the kits of the present invention contain a substantially isolated RSV antigen as a control. Preferably, the  
25 kits of the present invention further comprise a control antibody which does not react with the RSV antigen. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a RSV antigen (*e.g.*, the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which  
30 recognizes the first antibody may be conjugated to a detectable substrate). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized RSV antigen. The RSV antigen provided in the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above-described kit includes a solid support to which RSV antigen is attached. Such a kit may also include a non-attached

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reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the RSV antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing RSV antigens. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with a RSV antigen, and means for detecting the binding of the RSV antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound RSV antigen obtained by the methods of the present invention. After the RSV antigen binds to a specific antibody, the unbound serum components are removed by washing, reporter-labeled anti-human antibody is added, unbound anti-human antibody is removed by washing, and a reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-RSV antigen antibody on the solid support. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant RSV antigen, and a reporter-labeled anti-human antibody for detecting surface-bound anti-RSV antigen antibody.

#### **6. EXAMPLE: KINETIC ANALYSIS OF HUMANIZED RSV MABS BY BIACORE™**

A typical kinetic study involved the injection of 250 µl of monoclonal antibody ("Mab") at varying concentrations (25-300 nM) in PBS buffer containing 0.05% Tween-20

(PBS/Tween). The flow rate was maintained at 75  $\mu$ l/min, giving a 15 minute dissociation time. Following the injection of Mab, the flow was exchanged with PBS/Tween buffer for 30 min for determining the rate of dissociation. The sensor chip was regenerated between cycles with a 1 min pulse of 100 mM HCl. The regeneration step caused a minimal loss of binding capacity of the immobilized F-protein (4% loss per cycle). This small decrease did not change the calculated values of the rate constants for binding and dissociation (also called the  $k_{on}$  and  $k_{off}$ , respectively).

More specifically, for measurement of  $k_{assoc}$  (or  $k_{on}$ ), F protein was directly immobilized by the EDC/NHS method (EDC = N-ethyl-N'-[3-diethylaminopropyl]-carbodiimide). Briefly, 25 mg/ml of F protein in 10 mM NaoAc, pH 5.0 was prepared and about a 5-10  $\mu$ l injection gives about 30-50 RU (response units) of immobilized F protein under the above referenced conditions. The blank was subtracted for kinetic analysis. The column could be regenerated using 100 mM HCl (with 60 seconds of contact time being required for full regeneration). This treatment removed bound Fab completely without damaging the immobilized antigen and could be used for over 40 regenerations. For  $k_{on}$  measurements, Fab concentrations were 0.39 nM, .75 nM, 1.56 nM, 3.13 nM, 12.5 nM, 25 nM, 50 nM, and 100 nM. The dissociation phase was analyzed for approximately 900 seconds. Kinetics were analyzed by 1:1 Langmuir fitting (global fitting). Measurements were done in HBS-EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) Surfactant P20).

For measurements of combinatorial clones, as disclosed herein, the  $k_{on}$  and  $k_{off}$  were measured separately. The  $k_{on}$  was measured at conditions that were the same as those for the single mutation clones and was analyzed similarly.

For measuring  $k_{off}$ , the following conditions were employed. Briefly, 4100 RU of F protein were immobilized (as above) with CM-dextran used as the blank. Here, 3000 RU of Fab was bound (with dissociated Fab high enough to offset machine fluctuation). HBS plus 5 nM F protein (about 350 – 2000 times higher than the  $K_d$  – the dissociation equilibrium constant) was used as buffer. The dissociation phase was 6 – 15 hours at a flow rate of 5 ml/min. Under the conditions used herein, re-binding of the dissociated Fab was minimal. For further details, see the manual with the biosensor.

The binding of the high affinity anti-RSV antibodies to the F protein, or other epitopic sites on RSV, disclosed herein was calculated from the ratio of the first order rate constant for dissociation to the second order rate constant for binding or association ( $K_d = k_{off}/k_{on}$ ). The value for  $k_{on}$  was calculated based on the following rate equation:

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$$dR/dt = k_{on}[Mab]R_{max} - (k_{on}[Mab] + k_{off})R$$

where  $R$  and  $R_{max}$  are the response units at time  $t$  and infinity, respectively. A plot of  $dr/dt$  as a function of  $R$  gives a slope of  $(k_a[Mab] + k_d)$ --since these slopes are linearly related to the  $[Mab]$ , the value  $k_{on}$  can be derived from a replot of the slopes versus  $[Mab]$ . The slope of the new line is equal to  $k_{on}$ . Although the value of  $k_{off}$  can be extrapolated from the Y-intercept, a more accurate value was determined by direct measurement of  $k_{off}$ . Following the injection phase of the Mab, PBS/Tween buffer flows across the sensor chip. From this point,  $[Mab]=0$ . The above stated equation for  $dR/dt$  thus reduces to:

$$dr/dt = k \quad \text{or} \quad dR/R = k_{off} dt$$

Integration of this equation then gives:

$$\ln(R_0/R_t) = k_{off} t$$

where  $R_0/R_t$  are the response units at time 0 (start of dissociation phase) and  $t$ , respectively. Lastly, plotting  $\ln(R_0/R_t)$  as a function of  $t$  gives a slope of  $k_{off}$ .

The numerical values from such antibody variants were as shown in Tables 4-7 below.

Table 4. Summary of Kinetic Constants for High Potency Antibodies.

ANTIBODY	$K_{on} \times 10^5 (M^{-1}s^{-1})$	$K_{off} \times 10^{-4} (s^{-1})$	$EC_{50} (nM)$
**SYNAGIS®	2.04; 1.89; 2.18	7.64; 7.38; 7.02	3.57
**AFFF	1.08; 0.96; 1.24	2.74; 2.66; 2.06	
*1X-493L1FR	1.85	6.5	
*H3-3F4	4.59; 4.67; 5.72; 6.25; 5.33	4.45; 4.02	
*M3H9	6.05	3.38	
*Y10H6	7.57	4.62	
*DG	2.65; 2.83; 4.16; 3.18; 2.88	1.67; 4.44	

5	*AFFF	2.12; 1.56; 1.86	2.45; 4.46; 2.68	
	*6H8	3.14; 4.44	1.78; 4.73	
	*L1-7E5	3.29; 3.57; 4.05; 3.35; 4.26	1.92; 3.31; 2.29	
	*L2-15B10	3.69; 2.82; 3.12; 5.33; 3.78	1.34; 4.16; 2.70	
10	*P12f2	6.63	2.82	0.65
	*P12f4	5.27	2.99	0.70
	*P11d4	5.70; 5.72	7.17	>20
	*A1e9	7.9	4.53	2.5
15	*A12a6	7.43	2.30	0.62
	*A13a11	7.35	2.50	2.04
	*A13c4	7.81; 7.35	2.80	0.52

Table 5.

<u>Monoclonal Antibodies vs Bac-F (1:1)</u>					
		<b>Kon (x E+5)</b>	<b>Koff (x E-5)</b>	<b>KD (nM)</b>	<b>Chi2</b>
20	P12f2	4.07	12.8	0.31 (13)	0.9
	P12f4	4.95	5.55	0.11 (35)	0.6
	A13c4	3.00	3.96	0.13 (30)	1.2
	A12a6	4.60	1.65	0.04 (98)	1.2
25	A1e9	4.33	14.3	0.33 (12)	2.5
	A8c7	4.17	8.75	0.21 (19)	1.8
	P11d4	4.66	28.9	0.62 (6)	1.0
	A17d4	4.56	4.07	0.09 (43)	0.5
30	A4B4	4.34	1.06	0.02 (195)	1.5
	SYNAGIS®	1.32	51.5	3.90 (1)	0.6

35

Table 6.

**Monoclonal Antibodies vs NUF4 (1:1)**

	<b>K<sub>on</sub> (x E+5)</b>	<b>K<sub>off</sub> (x E-5)</b>	<b>K<sub>D</sub> (nM)</b>	<b>Chi2</b>
P12f2	5.41	17.8	0.33 (26)	1.2
P12f4	9.43	22.9	0.24 (36)	0.9
A13c4	3.65	27.2	0.75 (12)	1.8
A12a6	4.00	29.1	0.73 (12)	1.9
Ale9	8.43	58.4	0.69 (13)	0.9
A8c7	8.25	53.5	0.65 (13)	0.7
P11d4	9.04	76.6	0.85 (10)	2.5
A17d4	4.99	36.2	0.73 (12)	2.0
A4B4	4.96	28.2	0.57 (15)	1.9
SYNAGIS®	3.04	265	8.70 (1)	0.4

Table 7.

5 **Monoclonal Antibodies vs NUF4 (2:1)**

	<b>K<sub>on</sub> (x E+5)</b>	<b>K<sub>off</sub> (x E-5)</b>	<b>K<sub>D</sub> (nM)</b>	<b>Chi2</b>
P12f2	2.82	23.6	0.84 (371)	1.5
P12f4	2.73	63.6	2.33 (134)	4.9
A13c4	3.20	22.5	0.70 (446)	1.7
A12a6	2.18	40.8	1.87 (167)	1.9
Ale9	3.29	139	4.22 (74)	2.8
A8c7	4.30	114	2.65 (118)	2.0
P11d4	3.66	313	8.55 (36)	3.6
A17d4	2.64	29.2	1.11 (281)	1.7
A4B4	2.03	40.06	2.00 (156)	1.4
SYNAGIS®	0.78	2420	312(1)	1.3

1X-493L1FR, H3-3F4, M3H9, Y10H6, DG, AFFF, 6H8, L1-7E5, L2-15B10, P12f2, P12f4, P11d4, Ale9, A12a6, A13a11, A13c4 are Fab fragments having the framework sequences of Figure 2 and the indicated CDR sequences indicated listed in Table 2.

10 SYNAGIS® is a monoclonal antibody with the framework sequences of Figure 2 and constant regions as described in Johnson et al. (1997, Journal of Infectious Diseases 176:1215-1224) and U.S. Patent No. 5,824,307. The framework sequences of these antibodies may differ slightly from those of the Fab fragments.

The amino acid sequences of the indicated CDRs in Table 1 represent the amino acid residues located at the key locations within the CDRs of the high potency antibodies produced by the methods described herein and in copending applications Serial Nos. 60/168,426 and 60/186,252. For example, to increase the potency of an antibody by  
5 producing a higher  $k_{on}$  value, the amino acids located at the key positions as taught herein by the bold and underlined residues in Table 1 for the reference antibody would be replaced by the amino acids listed under CDRs in Table 2 (and also bold and underlined). Thus, these one letter codes represent the amino acids replacing the reference amino acids at the key positions (or critical positions) of the CDRs shown in Figure 2 (residues in bold in the  
10 sequences of Table 2) for a reference antibody whose potency is to be increased.

#### 7. **EXAMPLE: MICRONEUTRALIZATION ASSAY**

Neutralization of the antibodies of the present invention were determined by microneutralization assay. This microneutralization assay is a modification of the  
15 procedures described by Anderson et al. (1985, J. Clin. Microbiol. 22:1050-1052, the disclosure of which is hereby incorporated by reference in its entirety). The procedure used here is described in Johnson et al., 1999, J. Infectious Diseases 180:35-40, the disclosure of which is hereby incorporated by reference in its entirety. Antibody dilutions were made in triplicate using a 96-well plate. Ten TCID<sub>50</sub> of respiratory syncytial virus (RSV – Long  
20 strain) were incubated with serial dilutions of the antibody (or Fabs) to be tested for 2 hours at 37°C in the wells of a 96-well plate. RSV susceptible HEp-2 cells ( $2.5 \times 10^4$ ) were then added to each well and cultured for 5 days at 37°C in 5% CO<sub>2</sub>. After 5 days, the medium was aspirated and cells were washed and fixed to the plates with 80% methanol and 20% PBS. RSV replication was then determined by F protein expression. Fixed cells were  
25 incubated with a biotin-conjugated anti-F protein monoclonal antibody (pan F protein, C-site-specific MAb 133-1H) washed and horseradish peroxidase conjugated avidin was added to the wells. The wells were washed again and turnover of substrate TMB (thionitrobenzoic acid) was measured at 450 nm. The neutralizing titer was expressed as the antibody concentration that caused at least 50% reduction in absorbency at 450 nm (the  
30 OD<sub>450</sub>) from virus-only control cells. The results from the assay for the monoclonal antibodies and Fab fragments listed in Table 2 are shown in Table 4 *supra* and Table 8 *infra*.



Table 8. End Point RSV Microneutralization Titer Of High On Rate Mutant IgG and Fab

	<b>Molecule</b>	<b>Mean IC50 (Curve) μg/ml</b>	<b>STDEV Curve IC50</b>	<b>Fold Difference (Curve ICX50)</b>	<b>Mean IC50 (Control) μg/ml</b>	<b>STDEV Control IC50</b>	<b>Fold Difference (Control IC50)</b>	<b>n (assay repeat)</b>
5	**SYNAGIS®	0.4527	0.208	-	0.5351	0.238	-	8
	**A1e9	0.0625	0.0268	7	0.0645	0.0223	8	3
10	**A17d4	0.0342	0.022	13	0.0354	0.0187	15	4
	**P11d4	0.0217	0.0331	21	0.0289	0.0110	19	5
	**P12f2	0.0231	0.0141	20	0.0223	0.0083	24	6
15	**A8c7	0.0337	0.0309	13	0.0383	0.0283	14	5
	**A12a6	0.0357	0.0316	13	0.0354	0.0261	15	7
	**P12f4	0.0242	0.0163	19	0.0235	0.0076	23	7
20	**A13c4	0.0376	0.0268	12	0.0375	0.0213	14	6
	**A4B4	0.0171	0.0018	27	0.0154	0.00417	35	2
25	*A1e9	0.157	-	3	0.125	-	4	1
	*A17d4	0.0179	-	25	0.0171	-	31	1
	*P11d4	>1.00	-	-	>1.00	-	-	1
30	*P12f2	0.0407	0.0112	11	0.0326	0.00905	16	2
	*A8c7	0.177	-	3	0.157	-	34	1
35	*A12a6	0.0287	0.00417	16	0.0310	0.00982	17	2

	*P12f4	0.0464	0.00791	10	0.0351	0.0126	15	2
	*A13c4	0.0264	0.00141	17	0.0258	0.00071	21	2
5	*A4B4	0.0414	-	11	0.0411	-	13	1
	*A13a11	0.120	0.0222	4	0.1022	0.0260	5	2
10	*A1h5	0.194	0.462	2	0.176	0.0625	3	2

\*\* Monoclonal Antibody

\* Fab Fragment

### 8. EXAMPLE: RSV FUSION INHIBITION ASSAY

The ability of the antibodies of the invention or fragments thereof to block RSV-  
 15 induced fusion after viral attachment to the cells is determined in a fusion inhibition assay. This assay is identical to the microneutralization assay, except that the cells were infected with RSV (Long) for four hours prior to addition of antibody (Taylor et al, 1992, J. Gen. Virol. 73:2217-2223).

### 9. EXAMPLE: ISOTHERMAL TITRATION CALORIMETRY

Thermodynamic binding affinities and enthalpies were determined from isothermal  
 titration calorimetry (ITC) measurements on the interaction of antibodies with RSV F  
 glycoprotein (NUF4), an antigen which mimics the binding site of the RSV virus.

### Methods & Materials

#### Antibodies & Antigen

A13c4, A17d4, A4B4, and SYNAGIS® were diluted in dialysate and the  
 concentrations were determined by UV spectroscopic absorption measurements with a  
 Perkin-Elmer Lambda 4B Spectrophotometer using an extinction coefficient of 217,000 M<sup>-1</sup>  
 30 cm<sup>-1</sup> at the peak maximum at 280 nm. The diluted NUF4 concentrations were calculated  
 from the ratio of the mass of the original sample to that of the diluted sample since its  
 extinction coefficient was too low to determine an accurate concentration without  
 employing and losing a large amount of sample.

### ITC Measurements

The binding thermodynamics of the antibodies were determined from ITC measurements using a Microcal, Inc. VP Titration Calorimeter. The VP titration calorimeter consists of a matched pair of sample and reference vessels (1.409 ml) enclosed in an adiabatic enclosure and a rotating stirrer-syringe for titrating ligand solutions into the sample vessel. The ITC measurements were performed at 25°C and 35°C. The sample vessel contained the antibody in the phosphate buffer while the reference vessel contained just the buffer solution. The phosphate buffer solution was saline 67 mM PO<sub>4</sub> at pH 7.4 from HyClone, Inc. Five or ten µl aliquots of the 0.05 to 0.1 mM NUF4 solution were titrated 3 to 4 minutes apart into the antibody sample solution until the binding was saturated as evident by the lack of a heat exchange signal. With some antibody sample solutions, additional constant amounts of heat with the addition of each aliquot were observed following binding saturation of the antibody. This was attributed to a heat of dilution of the NUF4 titrant and was subtracted from the titrant heats obtained during the titration prior to analysis of the data.

A non-linear, least square minimization software program from Microcal, Inc., Origin 5.0, was used to fit the incremental heat of the *i*th titration ( $\Delta Q(i)$ ) of the total heat,  $Q_t$ , to the total titrant concentration,  $X_t$ , according to the following equations (I),

$$Q_t = nC_t \Delta H_b^\circ V \left\{ 1 + X_t/nC_t + 1/nK_b C_t - [(1 + X_t/nC_t + 1/nK_b C_t)^2 - 4X_t/nC_t]^{1/2} \right\} / 2 \quad (1a)$$

$$\Delta Q(i) = Q(i) + dV_i/2V \{Q(i) + Q(i-1)\} - Q(i-1) \quad (1b)$$

where  $C_t$  is the initial antibody concentration in the sample vessel,  $V$  is the volume of the sample vessel, and  $n$  is the stoichiometry of the binding reaction, to yield values of  $K_b$ ,  $\Delta H_b^\circ$ , and  $n$ . The optimum range of sample concentrations for the determination of  $K_b$  depends on the value of  $K_b$  and is defined by the following relationship.

$$C_t K_b n \leq 500 \quad (2)$$

so that at 1 µM the maximum  $K_b$  that can be determined is less than  $2.5 \times 10^8 \text{ M}^{-1}$ . If the first titrant addition did not fit the binding isotherm, it was neglected in the final analysis since it may reflect release of an air bubble at the syringe opening-solution interface.

### Results

The ITC results are summarized in Table 9. The higher than 2 stoichiometries in Table 9 indicate that either the concentration determination of the antibody or NUF4 was incorrect. Since the same NUF4 sample was used as a titrant with antibodies having the amino acid sequence of A13c4 at 35°C and A17d4 at 35°C, which exhibit in at least one of the titrations the correct stoichiometry of 2, it is assumed that the titrant concentration was correct and that the large values of n result from incorrectly determined antibody concentrations. However, it can be shown that the binding constants are critically dependent on the titrant concentration and, thus, despite the 2-3 disparity in n, the binding constants are correct. Since the binding constants of antibodies having the amino acid sequence of A4B4 and A13c4 at 25°C were near the upper determination limit by ITC (equation 2) and with the limited amount of available NUF4, it was decided to use 35°C as the reference temperature for comprising the binding affinities. The results summarized in Table 9 show that the binding affinities to NUF4 are in the order A4B4 > A13c4 > A17d4 > SYNAGIS®.

Table 9. Average Binding Constants and Enthalpies of NUF4 to Antibodies

Antibody	$K_b$	$\Delta H_b$ in kJ mol <sup>-1</sup>
A4B4	$269 \pm 74 \times 10^6 \text{ M}^{-1}$ or ~3.7 nM*	$92.8 \pm 1.0$
A13c4	$107 \pm 28 \times 10^6 \text{ M}^{-1}$ or 9 nM	$67 \pm 17$
A17d4	$75 \pm 14 \times 10^6 \text{ M}^{-1}$ or 13 nM	$68 \pm 10$
SYNAGIS®	$1.23 \pm 0.17 \times 10^6 \text{ M}^{-1}$ or 810 nM	$71 \pm 5$

\* Based only on the best titration run at 35°C.

4.0 nM is ITC lower limit of  $1/K_b$  range (ITC range is limited to  $[\text{antibody}]_n K_b = 500$  where n is the stoichiometry and  $[\text{antibody}]$  is the concentration of the antibody in the cell).

### 10. EXAMPLE: COTTON RAT PROPHYLAXIS

To determine the ability of SYNAGIS® to prevent lower respiratory tract RSV infection in cotton rats when administered by and intravenous (IV) route and to correlate the serum concentration of SYNAGIS® with a reduction in lung RSV titer.

### Materials & Methods

SYNAGIS® lot L94H048 was used for studies III-47 and III-47A. SYNAGIS® lot L95 K016 was used for study III-58. Bovine serum albumin (BSA) (fraction V, Sigma Chemicals). RSV-Long (A subtype) was propagated in Hep-2 cells.

- 5 On day 0, to groups of cotton rats (*Sigmodon hispidus*, average weight 100 g) were administered SYNAGIS®, RSV-IGIV or BSA was administered by intramuscular injection. Twenty-four hours post administration, the animals were bled and infected intranasally with  $10^5$  pfu of RSV. Twenty-four hours later, the animals were bled and infected intranasally with  $10^5$  PFU or RSV (Long Strain). Four days after the infection, animals were sacrificed,  
 10 and their lung tissue was harvested and pulmonary virus titers were determined by plaque titration. For studies III-47 and III-47A, the doses of monoclonal antibody ("MAb") consisted of 0.31, 0.63, 1.25, 2.5, 5.5 and 10 mg/kg (body weight). For studies III-58, the doses of MAb consisted of 0.63, 1.25, 2.5, 5.5 and 10 mg/kg (body weight). In all three studies bovine serum albumin (BSA) 10 mg/kg was used as a negative control. Human  
 15 antibody concentrations in the serum at the time of challenge are determined using a sandwich ELISA.

### Results

- The results of the individual experiments are presented in Tables 10-12. The results  
 20 of all of the experiments combined is shown in Table 13. All three studies show a significant reduction of pulmonary virus titers in animals treated with SYNAGIS®. A clear dose-response effect was observed in the animals. The combined data indicated that a dose of 2.5 mg/kg results in a greater than 99% reduction in lung RSV titer. The mean serum concentration of SYNAGIS® for this dose at the time of viral challenge was 28.6 µg/ml.

25

Table 10. EXPERIMENT III-47

	Compound	Number of Animals	Dose	Mean±Std Error Concentration of Human IgG (µg/ml)	Lung Viral Titer Geometric Mean ±Std Error (log10 pfu/gm)
30	BSA	4		0	$1.4 \times 10^5 \pm 1.7$
	SYNAGIS®	3	0.312mg/kg	$3.83 \pm 1.1$	$2.1 \times 10^4 \pm 2.1$
	SYNAGIS®	3	0.625mg/kg	$5.27 \pm 0.37$	$7.7 \times 10^4 \pm 1.6$
	SYNAGIS®	4	1.25mg/kg	$9.15 \pm 0.16$	$3.4 \times 10^4 \pm 1.3$
	SYNAGIS®	3	2.50mg/kg	$23.4 \pm 2.8$	$1.4 \times 10^3 \pm 1.7$
	SYNAGIS®	2	5.0mg/kg	$42.4 \pm 13.4$	$4.6 \times 10^2 \pm 4.6$
35	SYNAGIS®	4	10.0mg/kg	$141.1 \pm 14.4$	$1.0 \times 10^2 \pm 1.0$

Table 11. EXPERIMENT III-47A

	Compound	Number of Animals	Dose	Mean±Std Error Concentration of Human IgG (µg/ml)	Lung Viral Titer Geometric Mean ±Std Error (log10 pfu/gm)
5	BSA	4		0	$1.9 \times 10^5 \pm 1.2$
	SYNAGIS®	4	0.312mg/kg	$1.8 \pm 0.12$	$8.5 \times 10^4 \pm 1.2$
	SYNAGIS®	4	0.625mg/kg	$4.0 \pm 0.19$	$5.0 \times 10^4 \pm 1.6$
	SYNAGIS®	4	1.25mg/kg	$11.8 \pm 0.68$	$1.9 \times 10^3 \pm 1.4$
	SYNAGIS®	4	2.50mg/kg	$18.9 \pm 2.0$	$5.3 \times 10^3 \pm 1.6$
	SYNAGIS®	3	5.0mg/kg	$55.6 \pm 2.3$	$1.6 \times 10^2 \pm 1.3$
	SYNAGIS®	4	10.0mg/kg	$109.7 \pm 5.22$	$1.0 \times 10^2 \pm 1.0$

10

Table 12. EXPERIMENT III-58

	Compound	Number of Animals	Dose	Mean±Std Error Concentration of Human IgG (µg/ml)	Lung Viral Titer Geometric Mean ±Std Error (log10 pfu/gm)
15	BSA	4		0	$1.1 \times 10^5 \pm 1.2$
	SYNAGIS®	4	0.625mg/kg	$5.78 \pm 0.32$	$1.6 \times 10^4 \pm 1.2$
	SYNAGIS®	4	1.25mg/kg	$9.82 \pm 0.23$	$1.6 \times 10^3 \pm 1.3$
	SYNAGIS®	4	2.50mg/kg	$34.1 \pm 2.11$	$4.3 \times 10^2 \pm 1.6$
	SYNAGIS®	3	5.0mg/kg	$58.3 \pm 4.48$	$1.0 \times 10^2 \pm 1.0$
	SYNAGIS®	4	10.0mg/kg	$111.5 \pm 5.04$	$1.0 \times 10^2 \pm 1.0$

20

Table 13. III-47, III-47A and III-58 COMBINED

	Compound	Number of Animals	Dose	Mean±Std Error Concentration of Human IgG (µg/ml)	Lung Viral Titer Geometric Mean ±Std Error (log10 pfu/gm)
25	BSA	18		0	$1.3 \times 10^5 \pm 1.2$
	SYNAGIS®	7	0.312mg/kg	$2.67 \pm 0.60$	$4.6 \times 10^4 \pm 1.5$
	SYNAGIS®	17	0.625mg/kg	$5.27 \pm 0.27$	$2.7 \times 10^4 \pm 1.3$
	SYNAGIS®	18	1.25mg/kg	$10.1 \pm 0.29$	$3.3 \times 10^3 \pm 1.4$
	SYNAGIS®	17	2.50mg/kg	$28.6 \pm 2.15$	$9.6 \times 10^2 \pm 1.5$
	SYNAGIS®	15	5.0mg/kg	$55.6 \pm 3.43$	$1.3 \times 10^2 \pm 1.2$
	SYNAGIS®	18	10.0mg/kg	$117.6 \pm 5.09$	$1.0 \times 10^2 \pm 1.0$

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# 11. EXAMPLE: INTRAMUSCULAR COTTON RAT STUDIES

This experiment demonstrates that a greater reduction in RSV titer is achieved when A4b4, A4b4-F52S or A4b4/L1FR-S28R is administered intramuscularly to a cotton rat than when the same concentration of SYNAGIS® is administered intramuscularly to a cotton rat.

35

### **Materials & Methods**

#### **Intramuscular Cotton Rat Prophylaxis**

Cotton rats (*S. hispidus*, average weight 100 grams) were anesthetized with methoxyflurane and dosed with 0.1 ml of purified monoclonal antibody (MAb) or BSA control by intramuscular (i.m.) injection. Twenty-four hours later animals were again anesthetized, bled for serum MAb concentration determination, and challenged with  $10^5$  PFU RSV long by intranasal (i.n.) instillation. Four days later animals were sacrificed, serum samples were obtained, and their lungs were harvested. Lungs were homogenized in 10 parts (wt/vol) of Hanks Balanced Salt solution and the resultant suspension was used to determine pulmonary viral titers by plaque assay.

#### **Intramuscular Cotton Rat Pharmacokinetics**

Cotton rats (*S. hispidus*, average weight 100 grams) were anesthetized with methoxyflurane and dosed with 0.1 ml of purified MAb or BSA control by intramuscular (i.m.) injection. Twenty-four hours later all of the animals were bled for serum MAb concentration determination, and half of the animals from each group were sacrificed to perform bronchoalveolar lavage (BAL). Four days later the remaining animals were sacrificed, serum samples were obtained and BAL performed.

### **Results**

As shown in Tables 14-16, a greater reduction in RSV titer is achieved with equivalent or lower lung levels of A4b4, A4b4-F52S, or A4b4/L1FR-S28R as with SYNAGIS®.

Table 14: Intramuscular Cotton Rat Prophylaxis Data

	0.5mg/kg				0.125mg/kg			
	Serum IgG ( $\mu\text{g/ml}$ )	Lung IgG ( $\mu\text{g/ml}$ )	Virus Titer (pfu/gm)	log Virus Titer	Serum IgG ( $\mu\text{g/ml}$ )	Lung IgG ( $\mu\text{g/ml}$ )	Virus Titer (pfu/gm)	log Virus Titer
Synagis	3.4	0.099	$7.3 \times 10^3$	3.9	0.893	0.024	$3.1 \times 10^4$	4.5
A4b4-F52S	2.9	0.089	$7.3 \times 10^2$	2.9	0.781	0.020	$8.6 \times 10^3$	3.9
A4b4/L1FR-S28R	3.3	0.093	$6.1 \times 10^2$	2.8	0.748	0.016	$2.3 \times 10^4$	4.4
BSA			$5.9 \times 10^4$	4.8				

Table 15: Intramuscular Cotton Rat Prophylaxis Data

Molecule	0.5mg/kg			1mg/kg		
	Serum IgG μg/ml	Lung IgG μg/ml	Lung Virus log(10)	Serum IgG μg/ml	Lung IgG μg/ml	Lung Virus log(10)
A4b4	2.4	0.013	4.3	3.1	0.094	3.4
Synagis	1.9	0.038	4.4	4.2	0.212	3.3
BSA						4.4

Table 16: Intramuscular Cotton Rat Pharmacokinetics Data

Molecule	24 Hours		96 hours	
	Serum IgG (μg/ml)	BAL IgG (ng/ml)	Serum IgG (μg/ml)	BAL IgG (ng/ml)
A4b4	3.4	2.2	2.6	1.4
Synagis	4.1	5.3	2.8	3.5

## 12. EXAMPLE: *IN VITRO* ISOLATION OF SYNAGIS® SPECIFIC MONOCLONAL ANTIBODY RESISTANT MUTANTS(MARM) OF RSV STRAIN A/ LONG

This example demonstrates that MARMs can be isolated from RSV laboratory strain A/ Long upon multiple rounds of selection in the presence of SYNAGIS®.

### Materials:

SYNAGIS® (formulated product: ref RN1002.148), Control Pan RSV MAb Pool (Chemicon MAB858-4, a blend of three MAbs anti-F, G, and N proteins), anti-RSV Type A MAb (Chemicon MAB858-1), and anti-RSV Type B MAb (Chemicon MAB858-2) were used in this study. Secondary detecting reagents were either Alexa™488 conjugated Goat F(ab')<sub>2</sub> anti- Mouse or Human IgG (H+L). RSV A/ Long strain (5x10<sup>7</sup> TCID<sub>50</sub>/ml) was propagated from Master Virus Bank 031797. HEp-2 cells were propagated in EMEM supplemented with 10% FBS and 2 mM L-Gln in a 37°C, 5% CO<sub>2</sub> environment. Total cellular RNAs were isolated from infected HEp-2 cells with the Promega RNAgents Kit. cDNAs were synthesized with the Boehringer Mannheim 1<sup>st</sup> Strand cDNA Synthesis Kit, using Oligo- (dT) primer. Amplification of a fragment of the F protein for DNA sequencing was performed by Polymerase Chain Reaction (PCR), using gene-specific oligonucleotides (MR-120 and MR-122) and the Boehringer Mannheim High Fidelity PCR Kit.



### **Methods:**

#### **Selection of MARMs:**

- 4 x 10<sup>5</sup> HEp-2 cells were seeded per well of a 24-well plate in growth medium (EMEM, 10% FBS, 2 mM L-Gln) and incubated overnight in growth conditions (37°C, 5% CO<sub>2</sub>). 40 wells were seeded for individual MARM selection. Prior to infection, a fresh vial of master virus bank RSV A/ Long was thawed rapidly at 37°C and the virus titer adjusted to 4x10<sup>6</sup> pfu/ml in HEp-2 growth medium. SYNAGIS® was added to the virus inoculum at a final concentration of 30 µg/ml, and the mixture was incubated at 37°C for one hour. An aliquot of RSV was incubated with an irrelevant Human IgG1 Mab (MEDI-507) and was used as a negative control. An uninfected control well was also set up for each plate. Cells were washed once with fresh medium and were overlaid with 100 µl of RSV A/ Long virus stock/MAB mixture (multiplicity of infection [m.o.i.] = 4). Cells were incubated for four hours in growth conditions followed by addition of 1 ml of growth medium to each well. Cytopathic effect (CPE) was monitored on a daily basis by light microscopy.
- Following seven days of selection the contents of each well were supplemented with a further 30 µg/ml of SYNAGIS® or irrelevant Human IgG1 Mab, and were used to infect freshly seeded HEp-2 cells (4x10<sup>5</sup> cells/ well of 24-well plate). After a further seven days of selection the process was repeated one more time for a total of three rounds of selection.

#### **Plaque Purification of MARMs:**

- After the third round of selection, the contents of ten independent wells were chosen at random and used for plaque purification of MARMs. The remaining supernatants were mixed 1:1 with 50% sucrose solution and immediately frozen at -80°C. Supernatants were diluted 1:10, 1:100, and 1:1000 in growth medium and incubated with 30 µg/ml of SYNAGIS® for one hour prior to infection. 1 ml of virus inoculum was overlaid on monolayers of HEp-2 cells in 60mm round culture dishes (1x10<sup>6</sup> cells/ dish) and incubated for 4 hours in growth conditions. Following infection the inoculum was carefully aspirated and cells were overlaid with 3 ml growth medium supplemented with 0.8% low melting temperature agarose (Gibco BRL), and containing 30 µg/ml of SYNAGIS®. Dishes were returned to the incubator after complete solidification of agarose and monitored daily for plaque formation. Uninfected controls and wild type RSV control plates were set up for comparison. After five to six days, each plate was overlaid with an additional 2 ml of 0.8% agarose in growth medium supplemented with 50 µg/ml neutral red. Following an overnight incubation in growth conditions, plaques were scored and picked for a second round of purification.

Amplification of clonal MARMs:

Following a second round of plaque purification two clones from each isolate were expanded for production of high titer viral stocks. Individual plaques were picked with the aid of a pipet tip and incubated in 0.1 ml of fresh medium at 4°C overnight to elute the virus. Each aliquot was used to infect HEp-2 cells in flat bottom 96-well plates (1x10<sup>4</sup> cells/ well) in the presence of 30 µg/ml SYNAGIS®. After five days the entire well content was used to infect HEp-2 cells in the presence of 30 µg/ml of SYNAGIS® in 24-well plates. Each inoculum was subsequently expanded in the presence of 30 µg/ml of SYNAGIS® to a T-25 flask (1x10<sup>6</sup> cells/ flask) and 5 mls of high titer virus stock were produced. MARM stocks were frozen as described above.

F protein cDNA synthesis and DNA sequencing:

In order to determine the nucleotide sequence of an approximately 400 nucleotide region of the F protein gene thought to contain the epitope for SYNAGIS®, First strand cDNA synthesis was performed with total cellular RNAs isolated from MARM-infected HEp-2 cells at 4 days post-infection. Amplification of a fragment of the F protein for DNA sequencing was performed as described in Materials section. Amplified MARM F-protein cDNAs were purified by phenol/chloroform extraction and ethanol precipitation, and used in a PCR sequencing reaction with gene-specific oligos and Perkin-Elmer Cetus Big-Dye Terminator reaction mix to sequence the relevant region.

Immunofluorescence Assay (IFA):

Cells infected with RSV isolates were tested for anti-RSV binding by SYNAGIS® and Control Pan RSV MAbs Pool, as follows. Four to five day RSV-infected HEp-2 cultures were mixed with PBS and centrifuged at 300xg for 5 minutes at room temperature. Pellets were resuspended in a small volume of PBS for analysis. Five to ten microliters of each cell suspension were spotted per 5 mm well on acetone washed 12-well HTC super cured glass slides and allowed to air dry. Slides were fixed in cold (-20°C) acetone for 10 minutes. Reactions were blocked by spotting 10 µl of 1% BSA in PBS into each well and incubating for 10 minutes at room temperature. Slides were washed three times in 1X PBS/ 0.1% Tween-20 and air-dried. Ten microliters of each primary antibody reagent diluted to 250 ng/ml in blocking buffer were spotted per well and reactions were incubated in a humidified 37°C environment for 30 minutes. Slides were then washed for 1 minute in three changes of 1X PBS/ 0.1% Tween-20 and were air-dried. Ten microliters of appropriate secondary conjugated antibody reagent diluted to 250 ng/ml in blocking buffer were added to each

respective well and reactions were incubated in a humidified 37°C environment for an additional 30 minutes. Slides were then washed for 1 minute in three changes of 1X PBS/ 0.1% Tween-20. Five micro liters of 50% glycerol in PBS, 10 mM Tris, pH 8.0, 1 mM EDTA were spotted in each reaction well, and slides were mounted with cover slips. Each  
5 reaction well was subsequently analyzed by fluorescence microscopy at 200X power using a B-2A filter (EX 450-490 nm). Positive reactions were scored against an auto-fluorescent background obtained with unstained cells or cells stained with secondary reagent alone.

RSV positive reactions were characterized by bright fluorescence punctuated with small inclusions in the cytoplasm of infected cells.

10

#### Microneutralization Assay:

The procedure used here is described in Johnson et al., 1999, *J. Infectious Diseases* 180:35-40, the disclosure of which is hereby incorporated by reference in its entirety. Briefly, antibody dilutions were made in triplicate using a 96-well plate. Ten TCID<sub>50</sub> of  
15 RSV A MARMS were incubated with serial dilutions of the antibody to be tested for 2 hours at 37°C in the wells of a 96-well plate. The antibodies used in the assay comprised the heavy chain of SYNAGIS®, the heavy chain of SYNAGIS® with a point mutation or the heavy chain of A4B4, and the light chain of SYNAGIS®, the light chain of SYNAGIS® with point mutations, the light chain of A4B4 with point mutations, the light chain of L1FR (a.k.a. 1X-493L1FR) or the light chain of  
20 L1FR with point mutations. Mab 13/19 was used in the assay as a positive control. RSV susceptible HEp-2 cells (2.5 x 10<sup>4</sup>) were then added to each well and cultured for 5 days at 37°C in 5% CO<sub>2</sub>. After 5 days, the medium was aspirated and cells were washed and fixed to the plates with 80% methanol and 20% PBS. RSV replication was then determined by F  
25 protein expression. Fixed cells were incubated with a biotin-conjugated anti-F protein monoclonal antibody (pan F protein, C-site-specific MAb 133-1H) washed and horseradish peroxidase conjugated avidin was added to the wells. The wells were washed again and turnover of substrate TMB (thionitrobenzoic acid) was measured at 450 nm. The results from the assay for the monoclonal antibodies are shown in Table 19 *infra*.

30

#### **Results and Discussion:**

The binding activity of SYNAGIS® was tested by IFA against a panel of 20 RSV A/ Long MARMs obtained by three rounds of selection on HEp-2 cells in the presence of SYNAGIS®. A pool of monoclonal antibodies against the fusion, glycoprotein, and nuclear  
35 proteins of RSV (Control Pan RSV MAb Pool) was used as a positive control for detection

of RSV. Subtyping of RSV MARMs was performed with two monoclonal antibodies that distinguish type A and type B glycoproteins. As summarized in Table 17, lack of binding activity by SYNAGIS® was demonstrated in all 20 MARMs. Contrasting with lack of binding by SYNAGIS®, binding of the Control Pan RSV MAb Pool was demonstrated for all MARMs tested. All 20 MARMs were classified as RSV type A. Wild type RSV A/ Long infected HEp-2 cells bound SYNAGIS®, Pan RSV MAb Pool, and RSV Type A MAb, but failed to react with RSV Type B MAb, as expected.

DNA sequencing analysis of an approximately 400-nucleotide region of the RSV F protein cDNA encompassing the proposed SYNAGIS® epitope revealed a single mutation at the amino-acid level at position 272. Table 18 shows the amino acid change at position 272 in twelve isolates sequenced to date. Although the entire nucleotide sequence of RSV MARMs F protein has not been determined, these results suggest that amino acid 272 is a critical residue in modulating the binding of SYNAGIS® to its epitope.

The ability of various monoclonal antibodies to neutralize the replication of RSV A MARMs was determined. As shown in Table 19, the ability of the monoclonal antibodies to neutralize the replication of RSV MARMs varied depending upon the amino acid sequence of the heavy chain (HC) and light chain (LC) of the antibody.

Table 17. Characterization of anti-RSV Binding Activity by SYNAGIS®, Control Pan RSV MAb Pool (anti-F, G, N proteins), anti-RSV Type A MAb, and anti-RSV Type B MAb by Immunofluorescence Assay (IFA) on RSV A/ Long MARMs.

RSV MARM	Reactivity w/ SYNAGIS®	Reactivity w/ anti-RSV MAb Pool	Reactivity w/ anti-RSV type A MAb	Reactivity w/ Anti-RSV type B MAb
B1	-	+	+	-
B2	-	+	+	-
B3	-	+	+	-
B4	-	+	+	-
B5	-	+	+	-
B6	-	+	+	-
B7	-	+	+	-
B8	-	+	+	-
B9	-	+	+	-
B10	-	+	+	-
B11	-	+	+	-
B12	-	+	+	-
B13	-	+	+	-
B14	-	+	+	-
B15	-	+	+	-
B16	-	+	+	-
B17	-	+	+	-
B18	-	+	+	-

B19	-	+	+	-
B20	-	+	+	-
Wt RSV A/	+	+	+	-

5

Table 18. Amino acid sequence of a region of the wild type RSV A/ Long and MARMs F Protein encompassing the proposed SYNAGIS® epitope.

	Isolate	Amino Acid Sequence (Position number)													
	Wild type RSV Long	266	267	268	269	270	271	272	273	274	275	276	277	278	279
10	MARM B1	I	T	N	D	Q	K	N	L	M	S	N	N	V	Q
	MARM B2	I	T	N	D	Q	K	M	L	M	S	N	N	V	Q
	MARM B3	I	T	N	D	Q	K	M	L	M	S	N	N	V	Q
15	MARM B4	I	T	N	D	Q	K	M	L	M	S	N	N	V	Q
	MARM B6	I	T	N	D	Q	K	M	L	M	S	N	N	V	Q
	MARM B7	I	T	N	D	Q	K	T	L	M	S	N	N	V	Q
20	MARM B8	I	T	N	D	Q	K	M	L	M	S	N	N	V	Q
	MARM B9	I	T	N	D	Q	K	Q	L	M	S	N	N	V	Q
	MARM B10	I	T	N	D	Q	K	T	L	M	S	N	N	V	Q
25	MARM B13	I	T	N	D	Q	K	M	L	M	S	N	N	V	Q
	MARM B14	I	T	N	D	Q	K	Q	L	M	S	N	N	V	Q
	MARM B15	I	T	N	D	Q	K	M	L	M	S	N	N	V	Q
30															

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Table 19. MARM analysis by Microneutralization Assay

RSV Microneutralization Assay On RSV A MARMs to Synagis with RSV Monoclonal Antibody					
Amino Acid Change	K272Q	K272N	K272M	K272T	
Antibody	MARMs to SYNAGIS®				Note
HC/LC	MARM B9	MARM B1	MARM B2	MARM B7	
SYNAGIS®/SYNAGIS®	-	-	-	-	Purified Ab
A4B4/A4B4	+	+	+	+	Purified Ab
A4B4/SYNAGIS®	+	+	+	-	Purified Ab
SYNAGIS®/A4B4	-	-	-	-	Purified Ab
A4B4/L1FR	+	+	+	-	Purified Ab
A4B4/L1FR S28R	+	+	+	+	Purified Ab
A4B4/L1FR S28R,S52F	+	+	+	+	Purified Ab
A4B4/L1FR-28R, 52F, F93	-	-	-	-	Purified Ab
A4B4/A4b428S	+	+	+	+	Purified Ab
A4B4/A4b452S	+	+	+	+	Purified Ab
Mab 13/19	+	+	+	+	Purified Ab
SYNAGIS®/K53F	-	-	-	-	293H sup
SYNAGIS®/S28R	-	-	-	-	293H sup
SYNAGIS®/Q26S	-	-	-	-	293H sup
SYNAGIS®/A55D	-	-	-	-	293H sup
SYNAGIS®/K24S	-	-	-	-	293H sup
SYNAGIS®/C25A	-	-	-	-	293H sup
SYNAGIS®/L27S	-	-	-	-	293H sup

SYNAGIS®/S52F	-	-	-	-	-	-	293H sup
SYNAGIS®/L105V	-	-	-	-	-	-	293H sup
T98F/SYNAGIS®	-	-	-	-	-	-	Purified ab
S32A/SYNAGIS®	-	-	-	-	-	-	293H sup
S95D/SYNAGIS®	-	-	-	-	-	-	293H sup
D58H/SYNAGIS®	-	-	-	-	-	-	293H sup
A105Q/SYNAGIS®	-	-	-	-	-	-	293H sup
S65D/SYNAGIS®	-	-	-	-	-	-	293H sup
W100F/SYNAGIS®	-	-	-	-	-	-	293H sup

+ = neutralization detected; - = no neutralization detected

**13. EXAMPLE: *IN VITRO* ISOLATION OF A4B4 SPECIFIC  
MONOCLONAL ANTIBODY RESISTANT  
MUTANTS (MARM) OF RSV STRAIN A/ LONG**

This example demonstrates that MARMs can be isolated from RSV laboratory strain  
A/ Long upon multiple rounds of selection in the presence of A4B4

**Materials:**

A4B4 (Lot # 524-9, 3.57 mg /ml), Control Pan RSV MAb Pool (Chemicon  
MAB858-4, a blend of three MAbs anti-F, G, and N proteins), anti-RSV Type A MAb  
(Chemicon MAB858-1), and anti-RSV Type B MAb (Chemicon MAB858-2) were used in  
this study. Secondary detecting reagents were either Alexa<sup>TM</sup>488 conjugated Goat F (ab')<sub>2</sub>  
anti- Mouse or Human IgG (H+L). Virus bank of RSV A/ Long strain NWVB020500  
(2.38x10<sup>7</sup> TCID<sub>50</sub>/ml) propagated from Master Virus Bank 031797. HEp-2 cells were  
propagated in EMEM supplemented with 10% FBS and 2 mM L-Gln in a 37°C, 5% CO<sub>2</sub>  
environment. Messenger RNA was purified from the infected cells using the mRNA  
Capture Kit (Roche). The mRNA samples were used to make cDNA using the reagents  
from the cDNA First Strand Reaction Kit (Roche), followed by amplification of the RSV F  
protein gene by the Polymerase Chain Reaction (PCR) using gene specific primers.

**Methods:**

**Selection of MARMs:**

4 x 10<sup>5</sup> HEp-2 cells were seeded per well of a 24-well plate in growth medium  
(EMEM, 10% FBS, 2 mM L-Gln) and incubated overnight in growth conditions (37°C, 5%  
CO<sub>2</sub>). 44 wells were seeded for individual MARM selection. Prior to infection, a fresh vial  
of virus bank RSV A/ Long was thawed rapidly at 37°C and the virus titer adjusted to 4x10<sup>6</sup>  
pfu/ml in HEp-2 growth medium. A4B4 was added to the virus inoculum at a final  
concentration of 2 µg/ml, and the mixture was incubated at 37°C for one hour. An aliquot  
of RSV was incubated with an irrelevant Human IgG1 Mab (MEDI-507) and was used as a  
negative control. An uninfected control well was also set up for each plate. Cells were  
washed once with fresh medium and were overlaid with 100 µl of RSV A/ Long virus  
stock/MAB mixture. Cells were incubated for four hours in growth conditions followed by  
addition of 1 ml of growth medium to each well. Cytopathic effect (CPE) was monitored  
on a daily basis by light microscopy. Following seven days of selection the contents of each  
well were supplemented with a further 4 µg/ml of A4B4 or irrelevant Human IgG1 Mab,  
and were used to infect freshly seeded HEp-2 cells (4x10<sup>5</sup> cells/ well of 24-well plate).  
After a further seven days of selection the process was repeated one more time for a total of



three rounds of selection. The contents of wells showing clear CPE were mixed 1:1 with 50% sucrose solution and immediately frozen at  $-80^{\circ}\text{C}$

Plaque Purification of MARMs:

- 5           After the third round of selection, the contents of two independent wells were chosen at random and used for plaque purification of MARMs. A fresh vial of MARM stock (was frozen after third round of selection) was thawed at room temperature and was diluted 1:10, 1:100, and 1:1000 in growth medium and incubated with 4  $\mu\text{g/ml}$  of A4B4 for one hour prior to infection. 0.5 ml of virus inoculum was overlayed on monolayers of HEp-  
10 2 cells in 6 well plates ( $5 \times 10^5$  cells/ well) and incubated for 4 hours in growth conditions. Following infection the inoculum was carefully aspirated and cells were overlayed with 2 ml growth medium supplemented with 0.8% low melting temperature agarose (Gibco BRL), and containing 4  $\mu\text{g/ml}$  of A4B4. Dishes were returned to the incubator after complete solidification of agarose and monitored daily for plaque formation. Uninfected controls and  
15 wild type RSV control plates were set up for comparison. After five to six days, each plate was overlayed with an additional 2 ml of 0.8% agarose in growth medium supplemented with 50  $\mu\text{g/ml}$  neutral red. Following an overnight incubation in growth conditions, plaques were scored and picked for a second round of purification.

20 Amplification of clonal MARMs:

- Following a second round of plaque purification three clones from each isolate were expanded for production of high titer viral stocks. Individual plaques were picked with the aid of a pipet tip and incubated in 0.2 ml of fresh medium at  $4^{\circ}\text{C}$  overnight to elute the virus. Each aliquot was used to infect HEp-2 cells in flat bottom 24-well plates ( $2.5 \times 10^5$   
25 cells/ well- seeded day before) in the presence of 4  $\mu\text{g/ml}$  A4B4. After five days the entire well content was used to infect HEp-2 cells in the presence of 4  $\mu\text{g/ml}$  of A4b4 in 24-well plates. Each inoculum was subsequently expanded in the presence of 4  $\mu\text{g/ml}$  of A4b4 to a T-25 flask ( $6.5 \times 10^5$  cells/ flask – seeded day before) and 5 mls of high titer virus stock were produced. MARM stocks were frozen as described above.

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F protein cDNA synthesis and DNA sequencing:

- In order to determine the nucleotide sequence of an approximately 800 nucleotide region of the F protein gene thought to contain the epitope for SYNAGIS<sup>®</sup>, First strand cDNA synthesis was performed with mRNAs isolated from MARM-infected HEp-2 cells at  
35 4 days post-infection. RSV infected Hep2 cells were lysed in 150  $\mu\text{l}$  of lysis buffer

provided with the mRNA capture kit. Biotinylated oligo dT was diluted 1:10 with nuclease free H<sub>2</sub>O and 4 µl was added to each lysate. Samples were incubated 10 minutes at 42°C to allow the oligo dT to anneal to the mRNA. A 50 µl aliquot of the lysate was transferred to a streptavidin coated PCR tube and incubated for three minutes at 37°C. The lysates were  
5 removed from the PCR tubes and discarded. The RNA captured in the tubes was washed three times with 200 µl of wash buffer.

RT reactions were performed using reagents from the First Strand cDNA kit (Roche Molecular Biochemicals). A master mix was prepared so that each reaction contained 5 µl 10 X buffer, 5 µl dNTPs, 10 µl MgCl<sub>2</sub>, 1 µl gelatin, 2 µl RNase Inhibitor, 2 µl AMV-RT, in  
10 a final volume of 50 µl. Fifty microliter aliquots of the master mix were transferred to the PCR tubes containing the captured mRNA. Samples were placed in a thermocycler and incubated for two hours at 42°C. The cDNA reaction mix was then removed from the PCR tubes and discarded. The cDNA captured in the PCR tubes was washed with 200 µl of wash buffer. To obtain enough of the RSV F protein gene for sequence analysis, the cDNA  
15 was subjected to PCR using gene specific primers. Each reactions contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 200 µM dNTPs, 125 ng of each forward (5'AGTGTCTTAACCAGCAAAGTGTTAGA 3'; SEQ ID NO:258) and reverse (5' TCATTGACTTGAGATATTGATGCATC 3'; SEQ ID NO:259) primer, and 2.5 units of Taq polymerase (PE Biosystems) in a final volume of 50 µl. The temperature profile for all  
20 reactions was 95°C for 2 min, followed by 40 cycles of 95°C for 30 sec., 55°C for 45 sec, 72°C for 45 sec, with a final extension at 72°C for 10 minutes.

All PCR products were separated by electrophoresis on a 2% agarose gel in 1X TBE and visualized by ethidium bromide fluorescence.

PCR products were purified using Qiaquick spin columns (Qiagen and sequenced  
25 using the Big Dye-terminator PRISM kit (Applied Biosystems (ABI)). The reactions contained 70 ng of PCR product as template, 3 pmols of primer, and 8.0 µl of the PRISM dye-terminator reaction mix in a final volume of 20 µl. The reactions were subjected to thermal cycling according to ABI's dye-terminator sequencing instructions. Unincorporated dyes were removed from the extension products using Centri-Sep spin columns (Princeton  
30 Separations). Extension products were dried in a Savant Speed Vac and then dissolved in 10 µl HiDi Formamide (ABI) loading buffer. Samples were applied by electrophoresis in an ABI 3100 automated sequencer. Sequence data collected by the sequencer was analyzed using Lasargene (DNA Star).

Immunofluorescence Assay (IFA):

Cells infected with RSV isolates were tested for anti-RSV binding by A4B4, SYNAGIS® and Control Pan RSV MAb Pool, as follows. Four to five day RSV-infected HEp-2 cultures were mixed with PBS and centrifuged at 300xg for 5 minutes at room temperature. Pellets were resuspended in a small volume of PBS for analysis. Five to ten micro liters of each cell suspension were spotted per 5 mm well on acetone washed 12-well HTC supercured glass slides and allowed to air dry. Slides were fixed in cold (-20°C) acetone for 10 minutes. Reactions were blocked by spotting 10 µl of 1% BSA in PBS into each well and incubating for 10 minutes at room temperature. Slides were washed three times in 1X PBS/ 0.1% Tween-20 and air-dried. Ten micro liters of each primary antibody reagent diluted to 250 ng/ml in blocking buffer were spotted per well and reactions were incubated in a humidified 37°C environment for 30 minutes. Slides were then washed for 1 minute in three changes of 1X PBS/ 0.1% Tween-20 and were air-dried. Ten micro liters of appropriate secondary conjugated antibody reagent diluted to 250 ng/ml in blocking buffer were added to each respective well and reactions were incubated in a humidified 37°C environment for an additional 30 minutes. Slides were then washed for 1 minute in three changes of 1X PBS/ 0.1% Tween-20. Five micro liters of 50% glycerol in PBS, 10 mM Tris, pH 8.0, 1 mM EDTA were spotted in each reaction well, and slides were mounted with cover slips. Each reaction well was subsequently analyzed by fluorescence microscopy at 200X power using a B-2A filter (EX 450-490 nm). Positive reactions were scored against an auto fluorescent background obtained with unstained cells or cells stained with secondary reagent alone.

RSV positive reactions were characterized by bright fluorescence punctuated with small inclusions in the cytoplasm of infected cells.

Microneutralization Assay:

The procedure used here is described in Johnson et al., 1999, J. Infectious Diseases 180:35-40, the disclosure of which is hereby incorporated by reference in its entirety. Briefly, antibody dilutions were made in triplicate using a 96-well plate. Ten TCID<sub>50</sub> of RSV A MARMS were incubated with serial dilutions of the antibody to be tested for 2 hours at 37°C in the wells of a 96-well plate. The antibodies used in the assay comprised the heavy and light chain of SYNAGIS®, the heavy and light chain of A4b4, the combination of the heavy and light chain of SYNAGIS® and A4b4, the SYNAGIS® heavy chain or light chain with a point mutation in light chain or heavy chain, or A4b4 heavy chain with point mutations in light chain. Mab 13/19 was used as a positive control in the

assay. RSV susceptible HEP-2 cells ( $2.5 \times 10^4$ ) were then added to each well and cultured for 5 days at 37°C in 5% CO<sub>2</sub>. After 5 days, the medium was aspirated and cells were washed and fixed to the plates with 80% methanol and 20% PBS. RSV replication was then determined by F protein expression. Fixed cells were incubated with a biotin-conjugated anti-F protein monoclonal antibody (pan F protein, C-site-specific MAb 133-1H) washed and horseradish peroxidase conjugated avidin was added to the wells. The wells were washed again and turnover of substrate TMB (thionitrobenzoic acid) was measured at 450 nm. The results from the assay for the monoclonal antibodies are shown in Table 22 *infra*.

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### **Results and Discussion:**

The binding activity of A4B4 was tested by IFA against a panel of RSV A/ Long MARMs obtained by three rounds of selection on HEP-2 cells in the presence of SYNAGIS®. A pool of monoclonal antibodies against the fusion, glycoprotein, and nuclear proteins of RSV (Control Pan RSV MAb Pool) was used as a positive control for detection of RSV. Subtyping of RSV MARMs was performed with two monoclonal antibodies that distinguish type A and type B glycoproteins. As summarized in Table 20, lack of binding activity by A4B4 and SYNAGIS® was demonstrated in MARMs. Contrasting with lack of binding by A4B4 and SYNAGIS®, binding of the Control Pan RSV MAb Pool was demonstrated for all MARMs tested. Both MARMs were classified as RSV type A. Wild type RSV A/ Long infected HEP-2 cells bound to A4B4 and SYNAGIS®, Pan RSV MAb Pool, and RSV Type A MAb, but failed to react with RSV Type B MAb, as expected.

DNA sequencing analysis of an approximately 800-nucleotide region of the RSV F protein cDNA encompassing the proposed A4B4 epitope revealed mutation at the amino-acid level at position 272 and 276. Table 21 shows the amino acid change in isolates sequenced to date. Although the entire nucleotide sequence of RSV MARMs F protein has not been determined, these results suggest that amino acid 272 and 276 is a critical residue in modulating the binding of A4B4 to its epitope.

The ability of various monoclonal antibodies to neutralize the replication of a RSV A MARM was determined. As shown in Table 22, the ability of the monoclonal antibodies to neutralize the replication of a RSV MARM varied depending upon the amino acid sequence of the heavy chain (HC) and light chain (LC) of the antibody.

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Table 20. Characterization of anti-RSV Binding Activity by A4B4, Synagis®, Control Pan RSV MAb Pool (anti-F, G, N proteins), anti-RSV Type A MAb, and anti-RSV Type B MAb by Immunofluorescence Assay (IFA) on RSV A/ Long MARMs.

RSV MARM sample	Reactivity w/	Reactivity w/	Reactivity w/	Reactivity w/	Reactivity
	Synagis®	Anti-RSV MAb Pool	Anti-RSV type A MAb	Anti-RSV type B MAb	A4B4
MARM #6	-	+	+	-	-
MARM #9	-	+	+	-	-
MARM #10	-	+	+	-	-
MARM #11	-	+	+	-	-
Wt	+	+	+	-	+

Table 21. Amino acid sequence of a region of the wild type RSV A/ Long and MARMs F Protein.

Isolate	Amino Acid Sequence (Position number)													
	266	267	268	269	270	271	272	273	274	275	276	277	278	279
Wild type RSV	I	T	N	D	Q	K	K	L	M	S	N	N	V	Q
MARM #10	I	T	N	D	Q	K	E	L	M	S	Y	N	V	Q
MARM #6	I	T	N	D	Q	K	E	L	M	S	N	N	V	Q
MARM #9	I	T	N	D	Q	K	E	L	M	S	N	N	V	Q
MARM #11	I	T	N	D	Q	K	E	L	M	S	N	N	V	Q
MARM #14	I	T	N	D	Q	K	E	L	M	S	N	N	V	Q

Table 22. MARM analysis by Microneutralization Assay

<b>RSV Microneutralization Assay On RSV A MARMs to A4B4 with RSV Monoclonal Antibody</b>		
	<b>K272E, N276Y</b>	
<b>Antibody</b>	<b>MARM to</b>	<b>Note</b>
<b>HC/LC</b>	<b>MARM 10</b>	
SYNAGIS®/SYNAGIS®	-	Purified Ab
A4B4/A4B4	-	Purified Ab
A4b4/SYNAGIS®	-	Purified Ab
SYNAGIS®/A4B4	-	Purified Ab
A4B4/L1FR	-	Purified Ab
A4B4/L1FR S28R	-	Purified Ab
A4B4/L1FR S28R, S52F	-	Purified Ab
A4B4/L1FR-28R, 52F, 93F	-	Purified Ab
A4B4/A4B428S	-	Purified Ab
A4B4/A4B452S	-	Purified Ab
Mab 13/19	+	Purified Ab

+ = neutralization detected; - = no neutralization detected

**14. EXAMPLE: *IN VITRO* ISOLATION OF A4B4 SPECIFIC MONOCLONAL ANTIBODY RESISTANT MUTANTS (MARM) OF MARM B9( MARM OF RSV STRAIN A/ LONG TO SYNAGIS)**

This example demonstrates that MARMs can be isolated from MARM of RSV A long to Synagis (MARM B9) upon multiple rounds of selection in the presence of A4B4

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**Materials:**

A4B4 (Lot # 524-9, 3.57 mg /ml), Control Pan RSV MAb Pool (Chemicon MAB858-4, a blend of three MAbs anti-F, G, and N proteins), anti-RSV Type A MAb (Chemicon MAB858-1), and anti-RSV Type B MAb (Chemicon MAB858-2) were used in this study. Secondary detecting reagents were either Alexa™488 conjugated Goat F (ab')<sub>2</sub> anti- Mouse or Human IgG (H+L). MARM B9 (1.78×10<sup>6</sup> TCID<sub>50</sub>/ml) was used here. HEp-2 cells were propagated in EMEM supplemented with 10% FBS and 2 mM L-Gln in a 37°C, 5% CO<sub>2</sub> environment. Messenger RNA was purified from the infected cells using the mRNA Capture Kit (Roche). The mRNA samples were used to make cDNA using the reagents from the cDNA First Strand Reaction Kit (Roche), followed by amplification of

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the RSV F protein gene by the Polymerase Chain Reaction (PCR) using gene specific primers.

#### **Methods:**

##### **5 Selection of MARMs:**

- 4 x 10<sup>5</sup> HEp-2 cells were seeded per well of a 24-well plate in growth medium (EMEM, 10% FBS, 2 mM L-Gln) and incubated overnight in growth conditions (37°C, 5% CO<sub>2</sub>). 44 wells were seeded for individual MARM selection. Prior to infection, a fresh vial of virus bank MARM B9 was thawed rapidly at 37°C and the virus titer adjusted to 6x10<sup>5</sup> pfu/ml in HEp-2 growth medium. A4B4 was added to the virus inoculum at a final concentration of 2 µg/ml, and the mixture was incubated at 37°C for one hour. An aliquot of RSV was incubated with an irrelevant Human IgG1 Mab (MEDI-507) and was used as a negative control. An uninfected control well was also set up for each plate. Cells were washed once with fresh medium and were overlaid with 100 µl of RSV A/ Long virus stock/MAB mixture. Cells were incubated for four hours in growth conditions followed by addition of 1 ml of growth medium to each well. Cytopathic effect (CPE) was monitored on a daily basis by light microscopy. Following seven days of selection the contents of each well were supplemented with a further 4 µg/ml of A4B4 or irrelevant Human IgG1 Mab, and were used to infect freshly seeded HEp-2 cells (4x10<sup>5</sup> cells/ well of 24-well plate). After a further seven days of selection the process was repeated one more time for a total of three rounds of selection. The contents of wells showing clear CPE were mixed 1:1 with 50% sucrose solution and immediately frozen at -80°C

##### **Plaque Purification of MARMs:**

- After the third round of selection, the contents of independent wells were used for plaque purification of MARMs. A fresh vial of MARM stock (was frozen after third round of selection) was thawed at room temperature and was diluted 1:10, 1:100, and 1:1000 in growth medium and incubated with 4 µg/ml of A4B4 for one hour prior to infection. 0.5 ml of virus inoculum was overlayed on monolayers of HEp-2 cells in 6 well plates (5x10<sup>5</sup> cells/ well) and incubated for 4 hours in growth conditions. Following infection the inoculum was carefully aspirated and cells were overlayed with 2 ml growth medium supplemented with 0.8% low melting temperature agarose (Gibco BRL), and containing 4 µg/ml of A4B4. Dishes were returned to the incubator after complete solidification of agarose and monitored daily for plaque formation. Uninfected controls and MARM B9 RSV control plates were set up for comparison. After five to six days, each plate was overlayed with an additional 2

ml of 0.8% agarose in growth medium supplemented with 50 µg/ml neutral red. Following an overnight incubation in growth conditions, plaques were scored and picked for a second round of purification.

5 Amplification of clonal MARMs:

Following a second round of plaque purification three clones from isolate were expanded for production of high titer viral stocks. Individual plaques were picked with the aid of a pipet tip and incubated in 0.2 ml of fresh medium at 4°C overnight to elute the virus. Each aliquot was used to infect HEp-2 cells in flat bottom 24-well plates (2.5x10<sup>5</sup> cells/ well- seeded day before) in the presence of 4 µg/ml A4B4. After five days the entire well content was used to infect HEp-2 cells in the presence of 4 µg/ml of A4B4 in 24-well plates. Each inoculum was subsequently expanded in the presence of 4 µg/ml of A4B4 to a T-25 flask (6.5x10<sup>5</sup> cells/ flask – seeded day before) and 5 mls of high titer virus stock were produced. MARM stocks were frozen as described above.

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F protein cDNA synthesis and DNA sequencing:

In order to determine the nucleotide sequence of an approximately 800 nucleotide region of the F protein gene thought to contain the epitope for SYNAGIS®, First strand cDNA synthesis was performed with mRNAs isolated from MARM-infected HEp-2 cells at 4 days post-infection. RSV infected Hep2 cells were lysed in 150 µl of lysis buffer provided with the mRNA capture kit. Biotinylated oligo dT was diluted 1:10 with nuclease free H<sub>2</sub>O and 4 µl was added to each lysate. Samples were incubated 10 minutes at 42°C to allow the oligo dT to anneal to the mRNA. A 50 µl aliquot of the lysate was transferred to a streptavidin coated PCR tube and incubated for three minutes at 37°C. The lysates were removed from the PCR tubes and discarded. The RNA captured in the tubes was washed three times with 200 µl of wash buffer.

RT reactions were performed using reagents from the First Strand cDNA kit (Roche Molecular Biochemicals). A master mix was prepared so that each reaction contained 5 µl 10 X buffer, 5 µl dNTPs, 10 µl MgCl<sub>2</sub>, 1µl gelatin, 2 µl RNase Inhibitor, 2 µl AMV-RT, in a final volume of 50 µl. Fifty microliter aliquots of the master mix were transferred to the PCR tubes containing the captured mRNA. Samples were placed in a thermalcycler and incubated for two hours at 42°C. The cDNA reaction mix was then removed from the PCR tubes and discarded. The cDNA captured in the PCR tubes was washed with 200 µl of wash buffer To obtain enough of the RSV F protein gene for sequence analysis, the cDNA was subjected to PCR using gene specific primers. Each reactions contained 10 mM Tris-

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HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 200 μM dNTPs, 125 ng of each forward (5'AGTGTCTTAACCAGCAAAGTGTTAGA 3'; SEQ ID NO:258) and reverse (5'TCATTGACTTGAGATATTGATGCATC 3'; SEQ ID NO:259) primer, and 2.5 units of Taq polymerase (PE Biosystems) in a final volume of 50 μl. The temperature profile for all reactions was 95°C for 2 min, followed by 40 cycles of 95°C for 30 sec., 55°C for 45 sec, 72°C for 45 sec, with a final extension at 72°C for 10 minutes.

All PCR products were separated by electrophoresis on a 2% agarose gel in 1X TBE and visualized by ethidium bromide fluorescence.

PCR products were purified using Qiaquick spin columns (Qiagen and sequenced using the Big Dye-terminator PRISM kit (Applied Biosystems (ABI)). The reactions contained 70 ng of PCR product as template, 3 pmols of primer, and 8.0 μl of the PRISM dye-terminator reaction mix in a final volume of 20 μl. The reactions were subjected to thermal cycling according to ABI's dye-terminator sequencing instructions. Unincorporated dyes were removed from the extension products using Centri-Sep spin columns (Princeton Separations). Extension products were dried in a Savant Speed Vac and then dissolved in 10 μl HiDi Formamide (ABI) loading buffer. Samples were applied by electrophoresis in an ABI 3100 automated sequencer. Sequence data collected by the sequencer was analyzed using Lasargene (DNA Star).

#### 20 Immunofluorescence Assay (IFA):

Cells infected with RSV isolates were tested for anti-RSV binding by A4B4, SYNAGIS<sup>®</sup> and Control Pan RSV MAb Pool, as follows. Four to five day RSV-infected HEP-2 cultures were mixed with PBS and centrifuged at 300xg for 5 minutes at room temperature. Pellets were resuspended in a small volume of PBS for analysis. Five to ten micro liters of each cell suspension were spotted per 5 mm well on acetone washed 12-well HTC super cured glass slides and allowed to air dry. Slides were fixed in cold (-20°C) acetone for 10 minutes. Reactions were blocked by spotting 10 μl of 1% BSA in PBS into each well and incubating for 10 minutes at room temperature. Slides were washed three times in 1X PBS/ 0.1% Tween-20 and air-dried. Ten micro liters of each primary antibody reagent diluted to 250 ng/ml in blocking buffer were spotted per well and reactions were incubated in a humidified 37°C environment for 30 minutes. Slides were then washed for 1 minute in three changes of 1X PBS/ 0.1% Tween-20 and were air-dried. Ten micro liters of appropriate secondary conjugated antibody reagent diluted to 250 ng/ml in blocking buffer were added to each respective well and reactions were incubated in a humidified 37°C environment for an additional 30 minutes. Slides were then washed for 1 minute in three

changes of 1X PBS/ 0.1% Tween-20. Five micro liters of 50% glycerol in PBS, 10 mM Tris, pH 8.0, 1 mM EDTA were spotted in each reaction well, and slides were mounted with cover slips. Each reaction well was subsequently analyzed by fluorescence microscopy at 200X power using a B-2A filter (EX 450-490 nm). Positive reactions were scored against an auto fluorescent background obtained with unstained cells or cells stained with secondary reagent alone.

RSV positive reactions were characterized by bright fluorescence punctuated with small inclusions in the cytoplasm of infected cells.

#### 10 Microneutralization Assay:

The procedure used here is described in Johnson et al., 1999, J. Infectious Diseases 180:35-40, the disclosure of which is hereby incorporated by reference in its entirety. Briefly, antibody dilutions were made in triplicate using a 96-well plate. Ten TCID<sub>50</sub> of RSV A MARMS were incubated with serial dilutions of the antibody to be tested for 2 hours at 37°C in the wells of a 96-well plate. The antibodies used in the assay comprised the heavy chain of SYNAGIS® or the heavy chain of A4B4, and the light chain of SYNAGIS®, the light chain of A4B4, the light chain of A4B4 with a point mutation, the light chain of L1FR or the light chain of L1FR with point mutations. Mab 13/19 was used as a positive control in the assays. RSV susceptible HEp-2 cells ( $2.5 \times 10^4$ ) were then added to each well and cultured for 5 days at 37°C in 5% CO<sub>2</sub>. After 5 days, the medium was aspirated and cells were washed and fixed to the plates with 80% methanol and 20% PBS. RSV replication was then determined by F protein expression. Fixed cells were incubated with a biotin-conjugated anti-F protein monoclonal antibody (pan F protein, C-site-specific MAb 133-1H) washed and horseradish peroxidase conjugated avidin was added to the wells. The wells were washed again and turnover of substrate TMB (thionitrobenzoic acid) was measured at 450 nm. The results from the assay for the monoclonal antibodies are shown in Table 25 *infra*.

#### Results and Discussion:

The binding activity of A4B4 was tested by IFA against a MARM obtained by three rounds of selection on HEp-2 cells in the presence of A4B4. A pool of monoclonal antibodies against the fusion, glycoprotein, and nuclear proteins of RSV (Control Pan RSV MAb Pool) was used as a positive control for detection of RSV. Subtyping of RSV MARMS was performed with two monoclonal antibodies that distinguish type A and type B glycoproteins. As summarized in Table 23, lack of binding activity by A4B4 and

SYNAGIS® was demonstrated in both MARMs. Contrasting with lack of binding by A4B4 and SYNAGIS®, binding of the Control Pan RSV MAb Pool was demonstrated for all MARMs tested. Both MARMs were classified as RSV type A. Wild type RSV A/ Long infected HEp-2 cells bound to A4B4 and SYNAGIS®, Pan RSV MAb Pool, and RSV Type A MAb, but failed to react with RSV Type B MAb, as expected.

DNA sequencing analysis of an approximately 800 nucleotide region of the RSV F protein cDNA encompassing the proposed A4B4 epitope revealed mutation at the amino-acid level at position 272 and 262. Table 24 shows the amino acid change in isolates sequenced to date. Although the entire nucleotide sequence of RSV MARMs F protein has not been determined, these results suggest that amino acid 272 and 262 is a critical residue in modulating the binding of A4B4 to its epitope.

The ability of various monoclonal antibodies to neutralize the replication of RSV A MARMs was determined. As shown in Table 25, the ability of the monoclonal antibodies to neutralize the replication of RSV MARMs varied depending upon the amino acid sequence of the heavy chain (HC) and light chain (LC) of the antibody.

Table 23. Characterization of anti-RSV Binding Activity by A4B4, SYNAGIS®, Control Pan RSV MAb Pool (anti-F, G, N proteins), anti-RSV Type A MAb, and anti-RSV Type B MAb by Immunofluorescence Assay (IFA) on RSV A/ Long MARMs.

RSV MARM sample	Reactivity w/ Synagis®	Reactivity w/ Anti-RSV MAb Pool	Reactivity w/ Anti-RSV type A MAb	Reactivity w/ Anti-RSV type B MAb	Reactivity A4B4
MARM #13	-	+	+	-	-
B9	-	+	+	-	+
Wt	+	+	+	-	-

Table 24. Amino acid sequence of a region of the wild type RSV A/ Long and MARMs F Protein.

Isolate	Amino Acid Sequence (Position number)														
	262	266	267	268	269	270	271	272	273	274	275	276	277	278	279
Wild type RSV Long A	N	I	T	N	D	Q	K	K	L	M	S	N	N	V	Q
MARM #13	262	266	267	268	269	270	271	272	273	274	275	276	277	278	279
	K	I	T	N	D	Q	K	Q	L	M	S	N	N	V	Q

Table 25. MARM analysis by Microneutralization Assay

RSV Microneutralization Assay On RSV A MARMs to A4B4 with RSV Monoclonal Antibody_			
		K272Q,N262K	
Antibody		MARM to SYNAGIS® & A4B4	Note
HC/LC		MARM 13	
SYNAGIS®/SYNAGIS®		-	Purified Ab
A4B4/A4B4		-	Purified Ab
A4B4/SYNAGIS®		-	Purified Ab
SYNAGIS®/A4B4		-	Purified Ab
A4B4/L1FR		-	Purified Ab
A4B4/L1FR S28R		-	Purified Ab
A4B4/L1FR S28R, S52F		-	Purified Ab
A4B4/L1FR-28R, 52F, 93F		-	Purified Ab
A4B4/A4B428S		-	Purified Ab
A4B4/A4B452S		-	Purified Ab
Mab 13/19		+	Purified Ab

+ = neutralization detected; - = no neutralization detected

### 15. EXAMPLE: CLINICAL TRIALS

Antibodies of the invention or fragments thereof tested in *in vitro* assays and animal models may be further evaluated for safety, tolerance and pharmacokinetics in groups of normal healthy adult volunteers. The volunteers are administered intramuscularly, intravenously or by a pulmonary delivery system a single dose of 0.5 mg/kg, 3 mg/kg, 5 mg/kg, 10 mg/kg or 15 mg/kg of an antibody or fragment thereof which immunospecifically binds to a RSV antigen. Each volunteer is monitored at least 24 hours prior to receiving the single dose of the antibody or fragment thereof and each volunteer will be monitored for at least 48 hours after receiving the dose at a clinical site. Then volunteers are monitored as outpatients on days 3, 7, 14, 21, 28, 35, 42, 49, and 56 postdose.

Blood samples are collected via an indwelling catheter or direct venipuncture using 10 ml red-top Vacutainer tubes at the following intervals: (1) prior to administering the dose of the antibody or antibody fragment; (2) during the administration of the dose of the antibody or antibody fragment; (3) 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, and 48 hours after administering the dose of the antibody or antibody fragment; and (4) 3 days, 7 days 14 days, 21 days, 28 days, 35 days, 42 days, 49 days, and 56 days after administering the dose of the antibody or antibody fragment. Samples are allowed to clot at room temperature and serum will be collected after centrifugation.

The antibody or antibody fragment is partially purified from the serum samples and the amount of antibody or antibody fragment in the samples will be quantitated by ELISA. Briefly, the ELISA consists of coating microtiter plates overnight at 4°C with an antibody that recognizes the antibody or antibody fragment administered to the volunteer. The plates are then blocked for approximately 30 minutes at room temperature with PBS-Tween-0.5% BSA. Standard curves are constructed using purified antibody or antibody fragment, not administered to a volunteer. Samples are diluted in PBS-Tween-BSA. The samples and standards are incubated for approximately 1 hour at room temperature. Next, the bound antibody is treated with a labeled antibody (*e.g.*, horseradish peroxidase conjugated goat-anti-human IgG) for approximately 1 hour at room temperature. Binding of the labeled antibody is detected, *e.g.*, by a spectrophotometer.

The concentration of antibody or antibody fragment levels in the serum of volunteers are corrected by subtracting the predose serum level (background level) from the serum levels at each collection interval after administration of the dose. For each volunteer the pharmacokinetic parameters are computed according to the model-independent approach

(Gibaldi et al., eds., 1982, *Pharmacokinetics*, 2<sup>nd</sup> edition, Marcel Dekker, New York) from the corrected serum antibody or antibody fragment concentrations.

#### Equivalents

5           Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

10           All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

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The claims defining the invention are as follows:

1. An isolated antibody that immunospecifically binds to a respiratory syncytial virus (RSV) F antigen, wherein the antibody comprises:

- (a) a variable light (VL) chain comprising the amino acid sequence of SEQ ID NO:257;
- (b) a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (c) a variable heavy (VH) chain comprising the amino acid sequence of SEQ ID NO:256, and  
a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- (d) a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (e) a VH chain comprising the amino acid sequence of SEQ ID NO:256, and  
a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (f) a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- (g) a VL chain or VL domain comprising a VL complementary determining region (CDR) 1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6;
- (h) a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and  
a VL chain or VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6;
- (i) a VH chain comprising the amino acid sequence of SEQ ID NO:256; and  
a VL chain or VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6;
- (j) a VH domain comprising the amino acid sequence of SEQ ID NO:48; and  
a VL chain or VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of

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SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6;

- (k) a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and

a VL domain comprising the amino acid sequence of SEQ ID NO:76; or

- (l) a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and

a VL chain comprising the amino acid sequence of SEQ ID NO:257.

2. The antibody of claim 1, comprising:

a VH domain comprising the amino acid sequence of SEQ ID NO:48, and

a VL domain comprising the amino acid sequence of SEQ ID NO:76.

3. The antibody of claim 1, comprising:

a VH chain comprising the amino acid sequence of SEQ ID NO:256, and

a VL chain comprising the amino acid sequence of SEQ ID NO:257.

4. The antibody of claim 1, comprising:

a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and

a VL chain or VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6.

5. The antibody of any one of claims 1 to 4, wherein the antibody is a monoclonal antibody.

6. The antibody of claim 1, wherein the antibody is a Fab or F(ab')<sub>2</sub> fragment.

7. The antibody of any one of claims 1 to 4, wherein said antibody is a humanized antibody.

8. The antibody of claims 1 or 4, wherein the antibody comprises the framework regions in Figure 2.

9. A composition comprising the antibody of any one of the preceding claims and a pharmacologically acceptable carrier.



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10. The composition of claim 9, wherein the composition is a pharmaceutical composition.

11. An isolated nucleic acid molecule comprising a nucleotide sequence encoding:

- 5 (a) a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- (b) a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (c) a VH chain comprising the amino acid sequence of SEQ ID NO:256, and  
a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- 10 (d) a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (e) a VH chain comprising the amino acid sequence of SEQ ID NO:256, and  
a VL domain comprising the amino acid sequence of SEQ ID NO:76;
- (f) a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
a VL chain comprising the amino acid sequence of SEQ ID NO:257;
- 15 (g) a VL chain or VL domain comprising a VL complementary determining  
region (CDR) 1 having the amino acid sequence of SEQ ID NO:39, a VL  
CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3  
having the amino acid sequence of SEQ ID NO:6;
- 20 (h) a VH chain or VH domain comprising a VH CDR1 having the amino acid  
sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of  
SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID  
NO:20; and  
a VL chain or VL domain comprising a VL CDR1 having the amino acid  
sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of  
25 SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID  
NO:6;
- (i) a VH chain comprising the amino acid sequence of SEQ ID NO:256; and  
a VL chain or VL domain comprising a VL CDR1 having the amino acid  
sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of  
30 SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID  
NO:6;
- (j) a VH domain comprising the amino acid sequence of SEQ ID NO:48; and  
a VL chain or VL domain comprising a VL CDR1 having the amino acid  
sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of  
35 SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID  
NO:6;

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- (k) a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and  
 a VL domain comprising the amino acid sequence of SEQ ID NO:76; or  
 (l) a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and  
 a VL chain comprising the amino acid sequence of SEQ ID NO:257.
12. The nucleic acid molecule of claim 11, comprising a nucleotide sequence encoding:  
 a VH domain comprising the amino acid sequence of SEQ ID NO:48, and  
 a VL domain comprising the amino acid sequence of SEQ ID NO:76.
13. The nucleic acid molecule of claim 11, comprising a nucleotide sequence encoding:  
 a VH chain comprising the amino acid sequence of SEQ ID NO:256, and  
 a VL chain comprising the amino acid sequence of SEQ ID NO:257.
14. The nucleic acid molecule of claim 11, comprising a nucleotide sequence encoding:  
 a VH chain or VH domain comprising a VH CDR1 having the amino acid sequence of SEQ ID NO:10, a VH CDR2 having the amino acid sequence of SEQ ID NO:19, a VH CDR3 having the amino acid sequence of SEQ ID NO:20; and  
 a VL chain or VL domain comprising a VL CDR1 having the amino acid sequence of SEQ ID NO:39, a VL CDR2 having the amino acid sequence of SEQ ID NO:77, and a VL CDR3 having the amino acid sequence of SEQ ID NO:6.
15. A vector comprising the nucleic acid molecule of any one of claims 11 to 14.
16. The vector of claim 15, further comprising a nucleotide sequence which regulates the expression of the nucleic acid molecule.
17. A host cell genetically engineered to contain or express the nucleic acid molecule of any one of claims 11 to 14.
18. A host cell comprising the vector of claim 15 or 16.
19. The host cell of claim 17 or 18, wherein said host cell is a mammalian host cell.

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20. The host cell of claim 19, wherein the host cell is a mammalian cell selected from the group consisting of CHO, VERY, BHK, HeLa, COS, MDCK, 292, 3T3 W138, BT483, Hs578T, HTB2, BT20, T47D, NS0, CRL7030 and HsS78Bst.

21. A method for producing an antibody, comprising culturing the host cell of any one of claims 17-20 under conditions in which the nucleic acid molecule is expressed.

22. A kit comprising the antibody of any one of claims 1 to 8, in a container, and instructions for use.

23. A composition comprising the isolated nucleic acid of any one of claims 11 to 14 and a pharmaceutically acceptable carrier.

24. The composition of claim 23, wherein the composition is a pharmaceutical composition.

25. A kit comprising the isolated nucleic acid of any one of claims 11 to 14, in a container, and instructions for use.

26. A fusion protein comprising a non-IgG polypeptide recombinantly fused to the antibody of any one of claims 1 to 8.

27. A composition comprising the fusion protein of claim 26 and a pharmaceutically acceptable carrier.

28. A kit comprising the fusion protein of claim 26, in a container, and instructions for use.

29. An antibody conjugate comprising a non-IgG polypeptide chemically conjugated to the antibody of any one of claims 1 to 8.

30. The antibody conjugate of claim 29, wherein the non-IgG polypeptide is a diagnostic or therapeutic agent.

31. A composition comprising the antibody conjugate of claim 29 or 30 and a pharmaceutically acceptable carrier.

32. A kit comprising the antibody conjugate of claim 29 or 30, in a container, and instructions for use.

33. A method of producing the fusion protein of claim 26, comprising:

(a) transforming a host cell with a vector comprising a promoter operably linked to the nucleotide sequence encoding the fusion protein, and

(b) culturing said host cell to produce the fusion protein.

34. A method for detecting or diagnosing a RSV infection, comprising:

(a) contacting a cell or tissue sample of a subject with the antibody of any one of claims 1 to 8; and

(b) detecting the level of a RSV F antigen in the cell or the tissue sample;

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wherein an increase in the level of RSV F antigen the cell or tissue sample of the subject compared to a control level of RSV F antigen in a cell or tissue sample not infected with RSV is indicative of a RSV infection.

35. A method for detecting or diagnosing a RSV infection, comprising:

(a) contacting a cell or tissue sample of a subject with the fusion protein of claim 26; and

(b) detecting the level of a RSV F antigen in the cell or the tissue sample; wherein an increase in the level of RSV F antigen the cell or tissue sample of the subject compared to a control level of RSV F antigen in a cell or tissue sample not infected with RSV is indicative of a RSV infection.

36. A method for detecting or diagnosing a RSV infection, comprising:

(a) contacting a cell or tissue sample of a subject with the antibody conjugate of claim 28 or 29; and

(b) detecting the level of a RSV F antigen in the cell or the tissue sample; wherein an increase in the level of RSV F antigen the cell or tissue sample of the subject compared to a control level of RSV F antigen in a cell or tissue sample not infected with RSV is indicative of a RSV infection.

37. A sustained release formulation comprising the antibody according to any one of claims 1 to 8.

38. A composition comprising the antibody according to any one of claims 1 to 8 formulated for pulmonary delivery.

39. A composition comprising the antibody according to any one of claims 1 to 8 formulated for intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, or intranasal delivery.

40. A method for preventing a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the antibody of any one of claims 1 to 8.

41. A method for preventing a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the composition of claim 9 or 10.

42. A method of preventing a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the sustained release formulation of claim 37.

43. A method of preventing a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the composition of claim 39.

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44. A method of preventing a RSV infection or a symptom thereof in a mammal, comprising administering to the lungs of said mammal an effective amount of the composition of claim 38.

5 45. A method for treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the antibody of any one of claims 1 to 8.

46. A method for treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the composition of claim 9 or 10.

10 47. A method of treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the sustained release formulation of claim 37.

48. A method of treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to said mammal an effective amount of the composition of claim 39.

15 49. A method of treating or ameliorating a RSV infection or a symptom thereof in a mammal, comprising administering to the lungs of said mammal an effective amount of the composition of claim 38.

20 50. A method for reducing RSV titers in a mammal, comprising administering to said mammal an effective amount of the antibody of any one of claims 1 to 8.

51. A method for reducing RSV titers in a mammal, comprising administering to said mammal an effective amount of the composition of claim 9 or 10.

52. A method for reducing RSV titers in a mammal, comprising administering to said mammal an effective amount of the sustained release formulation of claim 37.

25 53. A method for reducing RSV titers in a mammal, comprising administering to said mammal an effective amount of the composition of claim 39.

54. A method for reducing RSV titers in a mammal, comprising administering to the lungs of said mammal an effective amount of the composition of claim 38.

30 55. The method according to any one of claims 40 to 42, 44 to 47, 49 to 52 or 54, wherein the antibody is administered by a nebulizer or inhaler.

56. The method according to any one of claims 40 to 43, 45 to 48 or 50 to 53, wherein the antibody is administered intradermally, intramuscularly, intraperitoneally, intravenously, subcutaneously, or intranasally.

35 57. The method according to any one of claims 40 to 56, wherein the mammal is a human subject.

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58. The method according to any one of claims 40 to 56, wherein the mammal is a human subject in an nursing home, a human subject in an orphanage, a human subject that has had a bone marrow transplant, an elderly human subject, or a human subject that has cystic fibrosis, bronchopulmonary dysplasia, congenital heart disease, congenital immunodeficiency or acquired immunodeficiency.

59. The method according to any one of claims 40 to 56, wherein the mammal is a human infant.

60. The method according to any one of claims 40 to 56, wherein the mammal is a human infant born prematurely or is at risk of hospitalization for a RSV infection.

61. The method according to any one of claims 40 to 60, comprising administering 15 mg/kg or less, 10 mg/kg or less, 5 mg/kg or less, or 1.5 mg/kg or less of the antibody to the mammal.

**Dated 25 January, 2008**  
**MedImmune, Inc.**

**Patent Attorneys for the Applicant/Nominated Person**  
**SPRUSON & FERGUSON**

A

DIQMTQSPST LSASVGDRVITCKCQLSVGYMH WYQQKPG 40  
CDR L1

KAPKLLIY DTSKLAS GVPSR FSGSGSGTEF TLTISSLOPD 80  
CDR L2

DFATYYC FQSGYPFT FGGGTKLEIK 106  
CDR L3

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSLSTSGMSVG WIR 40  
CDR H1

QPPGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80  
CDR H2

VLKVTNMDPA DTATYYCAR SMITNWFYDV W GAGTTVTVSS 120  
CDR H3

FIG. 1

A

DIQMTQSPST LSASVGDRVT ITCSASSSVGYMH WYQQKPG 40  
*CDR L1*

KAPKLLIY DTSKLAS GVPSR FSGSGSGTEF TLTISSLQPD 80  
*CDR L2*

DFATYYC FQGSGYPFT FGSG TKVEIK 106  
*CDR L3*

B

QVTLRESGPA LVKPTQTLTL TCTFSGFSL TSGMSVG WIR 40  
*CDR H1*

OPPGKALEWL A DIWWDDKKDYNPSLKS RLT ISKDTSKNQV 80  
*CDR H2*

VLKVTNMDPA DTATYYCAR SMITNWFYFDV WGQGTTVTVSS 120  
*CDR H3*

FIG. 2



## **EDITORIAL NOTE**

**APPLICATION NUMBER - 2002219944**

The following Sequence Listing pages **1** to **100** are part of the description. The claims pages follow on pages **153** to **160**.

## SEQUENCE LISTING

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 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
                   85                  90                  95  
  
 Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
                   100                  105                  110  
  
 Gly Thr Thr Val Thr Val Ser Ser  
                   115                  120

<210> 34  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 34  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 35  
 <211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 35  
 Asp Thr Phe Lys Leu Ser Ser  
 1 5

<210> 36  
 <211> 120  
 <212> PRT  
 <213> Homo sapiens

<400> 36  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> 37  
<211> 16  
<212> PRT  
<213> Homo sapiens

<400> 37  
Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser Leu Lys Asp  
1 5 10 15

<210> 38  
<211> 106  
<212> PRT  
<213> Homo sapiens

<400> 38  
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 39  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 39  
Ser Ala Ser Ser Arg Val Gly Tyr Met His  
1 5 10

<210> 40  
<211> 120  
<212> PRT  
<213> Homo sapiens

<400> 40  
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser  
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> 41  
<211> 16  
<212> PRT  
<213> Homo sapiens

<400> 41  
Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser Leu Lys Asp  
1 5 10 15

<210> 42  
<211> 106  
<212> PRT  
<213> Homo sapiens

<400> 42  
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 43  
<211> 7  
<212> PRT  
<213> Homo sapiens

<400> 43  
Asp Thr Met Tyr Gln Ser Ser  
1 5

<210> 44  
<211> 120

<212> PRT  
 <213> Homo sapiens  
  
 <400> 44  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser  
 115 120

<210> 45  
 <211> 16  
 <212> PRT  
 <213> Homo sapiens

<400> 45  
 Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser Leu Lys Asp  
 1 5 10 15

<210> 46  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 46  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Leu Pro Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Met Tyr Gln Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Phe Ser Gly Tyr Pro Phe Thr  
 85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
                   100                  105

<210> 47  
 <211> 10  
 <212> PRT  
 <213> Homo sapiens

<400> 47  
 Leu Pro Ser Ser Arg Val Gly Tyr Met His  
 1                  5                  10

<210> 48  
 <211> 120  
 <212> PRT  
 <213> Homo sapiens

<400> 48  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1                  5                  10                  15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
                   20                  25                  30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
                   35                  40                  45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
                   50                  55                  60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65                  70                  75                  80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
                   85                  90                  95

Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
                   100                  105                  110

Gly Thr Thr Val Thr Val Ser Ser  
                   115                  120

<210> 49  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 49  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1                  5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
                   20                  25                  30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
                   35                  40                  45

Asp Thr Phe Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
                   50                  55                  60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65                  70                  75                  80

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Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 50

<211> 7

<212> PRT

<213> Homo sapiens

<400> 50

Asp Thr Phe Phe Leu Asp Ser  
1 5

<210> 51

<211> 120

<212> PRT

<213> Homo sapiens

<400> 51

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser  
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> 52

<211> 106

<212> PRT

<213> Homo sapiens

<400> 52

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Arg Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60



Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> 53  
<211> 7  
<212> PRT  
<213> Homo sapiens

<400> 53  
Asp Thr Arg Tyr Gln Ser Ser  
1 5

<210> 54  
<211> 106  
<212> PRT  
<213> Homo sapiens

<220>  
<221> misc\_feature  
<223> VL Domain

<400> 54  
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> 55  
<211> 120  
<212> PRT  
<213> Homo sapiens

<400> 55  
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
                   35                  40                  45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
           50                  55                  60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
   65                  70                  75                  80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
                   85                  90                  95  
 Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Ala  
                   100                  105                  110  
 Gly Thr Thr Val Thr Val Ser Ser  
           115                  120

<210> 56  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> VL Domain

<400> 56  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
   1                  5                  10                  15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
           20                  25                  30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
           35                  40                  45  
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
   50                  55                  60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
   65                  70                  75                  80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
                   85                  90                  95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
           100                  105

<210> 57  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> VL Domain

<400> 57  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
   1                  5                  10                  15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
                   20                  25                  30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
                   35                  40                  45  
 Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
                   50                  55                  60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
                   65                  70                  75                  80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
                   85                  90                  95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
                   100                  105

<210> 58  
 <211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 58  
 Asp Thr Tyr Lys Gln Thr Ser  
 1                  5

<210> 59  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> VL Domain

<400> 59  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1                  5                  10                  15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
                   20                  25                  30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
                   35                  40                  45  
 Asp Thr Arg Tyr Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
                   50                  55                  60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
                   65                  70                  75                  80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
                   85                  90                  95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
                   100                  105

<210> 60  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 60  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Phe Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 61  
 <211> 9  
 <212> PRT  
 <213> Homo sapiens

<400> 61  
 Phe Gln Gly Ser Phe Tyr Pro Phe Thr  
 1 5

<210> 62  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> VL Domain

<400> 62  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 63  
 <211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 63  
 Asp Thr Phe Lys Leu Thr Ser  
 1 5

<210> 64  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> VL Domain

<400> 64  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
 100 105

<210> 65  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 65  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> 66  
<211> 7  
<212> PRT  
<213> Homo sapiens

<400> 66  
Asp Thr Phe Arg Leu Ala Ser  
1 5

<210> 67  
<211> 120  
<212> PRT  
<213> Homo sapiens

<400> 67  
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
1 5 10 15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
20 25 30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
50 55 60

Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
65 70 75 80

Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
85 90 95

Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Ala  
100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
115 120

<210> 68  
<211> 106  
<212> PRT  
<213> Homo sapiens

<400> 68  
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Tyr Arg His Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> 69  
<211> 7  
<212> PRT  
<213> Homo sapiens

<400> 69  
Asp Thr Tyr Arg His Ser Ser  
1 5

<210> 70  
<211> 106  
<212> PRT  
<213> Homo sapiens

<400> 70  
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> 71  
<211> 106  
<212> PRT  
<213> Homo sapiens

<400> 71  
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Ser Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Phe Phe His Arg Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

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Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys  
100 105

<210> 72  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 72  
Ser Leu Ser Ser Ser Val Gly Tyr Met His  
1 5 10

<210> 73  
<211> 7  
<212> PRT  
<213> Homo sapiens

<400> 73  
Asp Thr Phe Phe His Arg Ser  
1 5

<210> 74  
<211> 106  
<212> PRT  
<213> Homo sapiens

<400> 74  
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
35 40 45

Asp Thr Leu Leu Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105

<210> 75  
<211> 7  
<212> PRT  
<213> Homo sapiens

<400> 75  
Asp Thr Leu Leu Leu Asp Ser  
1 5



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<210> 76  
 <211> 106  
 <212> PRT  
 <213> Homo sapiens

<400> 76  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Ser Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
 100 105

<210> 77  
 <211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 77  
 Asp Thr Ser Phe Leu Asp Ser  
 1 5

<210> 78  
 <211> 120  
 <212> PRT  
 <213> Homo sapiens

<400> 78  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Ala  
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser  
           115                  120

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Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser		
145					150					155					160		
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val		
				165					170					175			
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro		
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Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys		
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Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp		
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Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly		
225					230					235					240		
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile		
				245					250					255			
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu		
			260					265					270				
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His		
		275					280					285					
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg		
	290					295					300						
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys		
305					310					315					320		
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu		
				325					330					335			
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr		
			340					345					350				
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu		
		355					360					365					
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp		
	370					375					380						
Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val		
385					390					395					400		
Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp		
				405					410					415			
Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His		
			420					425					430				
Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro		
		435					440					445					
Gly	Lys																
	450																

<210> 209  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 209  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Lys Cys Gln Leu Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 210  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 210  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Ser Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 211  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens  
 <400> 211  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Phe Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210



<210> 212  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 212  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445

Gly Lys  
 450

<210> 213  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 213  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Tyr Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210  
 <210> 214  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens  
 <400> 214  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450

<210> 215  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 215  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Arg Gly Leu Pro Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 216  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 216  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Pro  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 217  
 <211> 213

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 217

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Pro Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Met Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

&lt;210&gt; 218

&lt;211&gt; 450

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 218

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp	Leu	Ala	Asp	Ile	Trp	Trp	Asp	Gly	Lys	Lys	His	Tyr	Asn	Pro	Ser
50						55					60				
Leu	Lys	Asp	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Thr	Ser	Lys	Asn	Gln	Val
65					70					75					80
Val	Leu	Lys	Val	Thr	Asn	Met	Asp	Pro	Ala	Asp	Thr	Ala	Thr	Tyr	Tyr
				85					90					95	
Cys	Ala	Arg	Asp	Met	Ile	Phe	Asn	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val
		115					120					125			
Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala
	130					135					140				
Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser
145					150					155					160
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val
				165					170					175	
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro
			180					185					190		
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys
		195					200					205			
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp
	210					215					220				
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly
225					230					235					240
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile
				245					250					255	
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu
			260					265					270		
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His
		275					280					285			
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg
	290					295					300				
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys
305					310					315					320
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu
				325					330					335	
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr
			340					345					350		
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu
		355					360					365			
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp
	370					375					380				



Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 219  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens  
 <400> 219  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 220  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 220  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445

Gly Lys  
 450

<210> 221  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 221  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210  
 <210> 222  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens  
 <400> 222  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450

<210> 223  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 223  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 224  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 224  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys Ser Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140

Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	145	150	155	160
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	165	170	175	
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	180	185	190	
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	195	200	205	
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	210	215	220	
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	225	230	235	240
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	245	250	255	
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	260	265	270	
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	275	280	285	
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	290	295	300	
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	305	310	315	320
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	325	330	335	
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	340	345	350	
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	355	360	365	
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	370	375	380	
Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	385	390	395	400
Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	405	410	415	
Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	420	425	430	
Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	435	440	445	
Gly	Lys															450			
<210>	225																		
<211>	213																		

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 225

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Leu Pro Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Met Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

&lt;210&gt; 226

&lt;211&gt; 450

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 226

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45



Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 227  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens  
 <400> 227  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 228  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 228  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Ser Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450

<210> 229  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 229  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Pro Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Arg Tyr Gln Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210  
 <210> 230  
 <211> 451  
 <212> PRT  
 <213> Homo sapiens  
 <400> 230  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser  
 20 25 30  
 Gly Met Ser Val Gly Tyr Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu  
 35 40 45  
 Glu Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro  
 50 55 60  
 Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln  
 65 70 75 80  
 Val Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr  
 85 90 95  
 Tyr Cys Ala Arg Ser Met Ile Thr Asn Trp Tyr Phe Asp Val Trp Gly  
 100 105 110  
 Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125  
 Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
 130 135 140  
 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160  
 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175  
 Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
 180 185 190  
 Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
 195 200 205  
 Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys  
 210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly  
 225 230 235 240  
 Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met  
 245 250 255  
 Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His  
 260 265 270  
 Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val  
 275 280 285  
 His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr  
 290 295 300  
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly  
 305 310 315 320  
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile  
 325 330 335  
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val  
 340 345 350  
 Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser  
 355 360 365  
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu  
 370 375 380  
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro  
 385 390 395 400  
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val  
 405 410 415  
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met  
 420 425 430  
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser  
 435 440 445  
 Pro Gly Lys  
 450

<210> 231  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 231  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 232  
 <211> 450  
 <212> PRT  
 <213> HOMO sapiens

<400> 232  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 233  
 <211> 213



&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 233

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Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1      5      10      15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met
      20      25      30

His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
      35      40      45

Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
      50      55      60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp
65      70      75      80

Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr
      85      90      95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
      100      105      110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
      115      120      125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
      130      135      140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145      150      155      160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
      165      170      175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
      180      185      190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
      195      200      205

Asn Arg Gly Glu Cys
      210

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&lt;210&gt; 234

&lt;211&gt; 450

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 234

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Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1      5      10      15

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala
      20      25      30

Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
      35      40      45

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Trp	Leu	Ala	Asp	Ile	Trp	Trp	Asp	Asp	Lys	Lys	Asp	Tyr	Asn	Pro	Ser
50						55					60				
Leu	Lys	Ser	Arg	Leu	Thr	Ile	Ser	Lys	Asp	Thr	Ser	Lys	Asn	Gln	Val
65					70					75					80
Val	Leu	Lys	Val	Thr	Asn	Met	Asp	Pro	Ala	Asp	Thr	Ala	Thr	Tyr	Tyr
				85					90					95	
Cys	Ala	Arg	Asp	Met	Ile	Phe	Asn	Trp	Tyr	Phe	Asp	Val	Trp	Gly	Gln
			100					105					110		
Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val
		115					120					125			
Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala
130						135					140				
Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser
145					150					155					160
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val
				165					170					175	
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro
			180					185					190		
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys
		195					200					205			
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp
	210					215					220				
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly
225					230					235					240
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile
				245					250					255	
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu
			260					265					270		
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His
		275					280					285			
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg
	290					295					300				
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys
305					310					315					320
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu
				325					330					335	
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr
			340					345					350		
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu
		355					360					365			
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp
	370					375					380				

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 235  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens  
 <400> 235  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Tyr Lys Gln Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 236  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 236  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445

Gly Lys  
450

<210> 237  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 237  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Arg Tyr Leu Ser Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210  
 <210> 238  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens  
 <400> 238  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450

<210> 239  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 239  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 240  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 240  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Ser Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140



Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 241  
 <211> 213

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 241

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Phe Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

&lt;210&gt; 242

&lt;211&gt; 450

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 242

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 243  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens  
 <400> 243  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
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<210> 244  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 244  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
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 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 245  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens  
 <400> 245  
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 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210  
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 <211> 450  
 <212> PRT  
 <213> Homo sapiens  
 <400> 246  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Cln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Thr Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450

<210> 247  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 247  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Arg Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60



Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 248  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 248  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Ser Met Ile Thr Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140

Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	
145					150					155					160	
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	
				165					170					175		
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	
			180					185					190			
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	
		195					200					205				
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	
	210					215					220					
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	
225					230					235					240	
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	
				245					250					255		
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	
			260					265						270		
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	
		275					280					285				
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	
	290					295					300					
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	
305					310					315					320	
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	
				325					330					335		
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	
			340					345					350			
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	
		355					360					365				
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	
	370					375					380					
Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	
385					390					395					400	
Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	
				405					410					415		
Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	
			420					425					430			
Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	
		435					440					445				
Gly	Lys															
	450															
<210>	249															
<211>	214															

<212> PRT  
 <213> Homo sapiens  
  
 <400> 249  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Pro Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Tyr Arg His Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly  
 50 55 60  
 Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 65 70 75 80  
 Asp Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe  
 85 90 95  
 Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
 100 105 110  
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
 115 120 125  
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
 130 135 140  
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
 145 150 155 160  
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
 165 170 175  
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
 180 185 190  
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
 195 200 205  
 Phe Asn Arg Gly Glu Cys  
 210

<210> 250  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens  
  
 <400> 250  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45

Trp Leu Ala Asp Ile Trp Trp Asp Gly Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Trp Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450  
 <210> 251  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens  
 <400> 251  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Leu Ser Ser Ser Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Phe Phe His Arg Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 252  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 252  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220  
 Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450

<210> 253  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 253  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Leu Leu Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210  
 <210> 254  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens  
 <400> 254  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140  
 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205  
 Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp  
 210 215 220



Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly  
 225 230 235 240  
 Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile  
 245 250 255  
 Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu  
 260 265 270  
 Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His  
 275 280 285  
 Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg  
 290 295 300  
 Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys  
 305 310 315 320  
 Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu  
 325 330 335  
 Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr  
 340 345 350  
 Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu  
 355 360 365  
 Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp  
 370 375 380  
 Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val  
 385 390 395 400  
 Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp  
 405 410 415  
 Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His  
 420 425 430  
 Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro  
 435 440 445  
 Gly Lys  
 450

<210> 255  
 <211> 213  
 <212> PRT  
 <213> Homo sapiens

<400> 255  
 Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

<210> 256  
 <211> 450  
 <212> PRT  
 <213> Homo sapiens

<400> 256  
 Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln  
 1 5 10 15  
 Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ala  
 20 25 30  
 Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu  
 35 40 45  
 Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys His Tyr Asn Pro Ser  
 50 55 60  
 Leu Lys Asp Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val  
 65 70 75 80  
 Val Leu Lys Val Thr Asn Met Asp Pro Ala Asp Thr Ala Thr Tyr Tyr  
 85 90 95  
 Cys Ala Arg Asp Met Ile Phe Asn Phe Tyr Phe Asp Val Trp Gly Gln  
 100 105 110  
 Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
 115 120 125  
 Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140

Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	145	150	155	160
Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	165	170	175	
Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	180	185	190	
Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	195	200	205	
Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	210	215	220	
Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	225	230	235	240
Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	245	250	255	
Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	260	265	270	
Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	275	280	285	
Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	290	295	300	
Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	305	310	315	320
Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	325	330	335	
Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	340	345	350	
Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	355	360	365	
Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	370	375	380	
Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	385	390	395	400
Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	405	410	415	
Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	420	425	430	
Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	435	440	445	
Gly	Lys															450			
<210>	257																		
<211>	213																		

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 257

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly  
 1 5 10 15  
 Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Arg Val Gly Tyr Met  
 20 25 30  
 His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
 35 40 45  
 Asp Thr Ser Phe Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
 50 55 60  
 Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Asp  
 65 70 75 80  
 Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly Ser Gly Tyr Pro Phe Thr  
 85 90 95  
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110  
 Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125  
 Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys  
 130 135 140  
 Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160  
 Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175  
 Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190  
 Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205  
 Asn Arg Gly Glu Cys  
 210

&lt;210&gt; 258

&lt;211&gt; 26

&lt;212&gt; DNA

&lt;213&gt; Artificial

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Primer

&lt;400&gt; 258

agtgtcttaa ccagcaaagt gttaga

26

&lt;210&gt; 259

&lt;211&gt; 26

&lt;212&gt; DNA

&lt;213&gt; Artificial

<220>

<223> Description of Artificial Sequence: Primer

<400> 259

tcattgactt gagatattga tgcata

26